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PRACTICAL PHYSIOLOGICAL CHEMISTRY

BY

R. H. ADERS PLIMMER, D.Sc.

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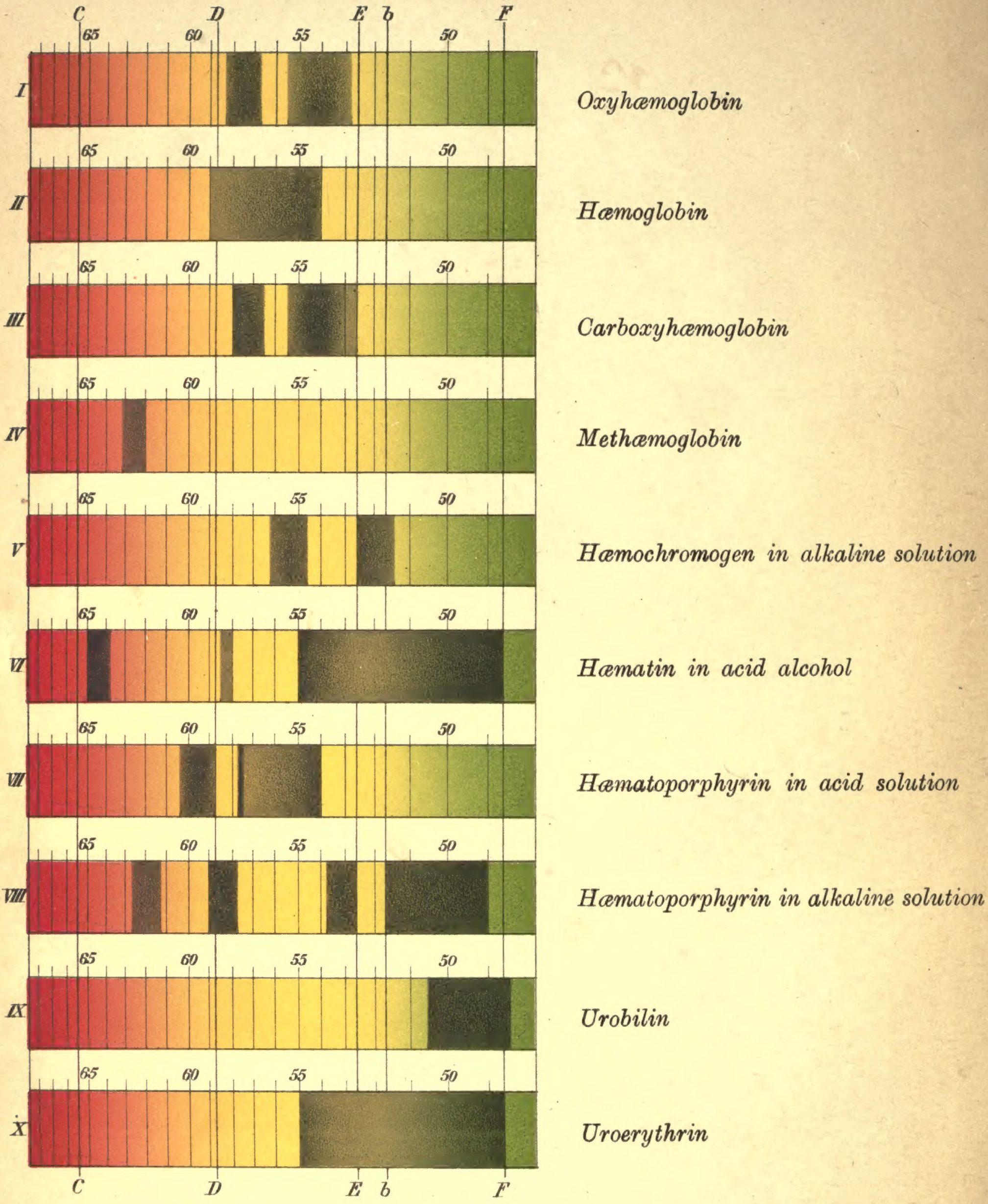

ABSORPTION SPECTRA

(From Hoppe-Seyler-Thierfelder's "*Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse.*")

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PREFACE

THIS book was originally compiled as a handbook for practical work in Physiological Chemistry at University College, London, since no single text or class book covered the complete course, or treated Physiological Chemistry as part of the subject of Organic Chemistry, or even as an independent subject.

The present book must still be regarded mainly as a compilation. It represents an attempt to give to the worker a nearly complete statement of the whole subject. Each section has a short explanatory summary of the essential points, so as to connect the various sections together. The essential points are illustrated by the practical experiments, which are printed in different type.

The illustrations are also compiled from various sources. These are mentioned underneath each figure. The illustrations of apparatus not so mentioned have been drawn from my own sketches. For those of the osazone crystals, haemin, and tyrosine, I am indebted to Miss V. G. Sheffield, who has also kindly helped in reading the proof sheets.

In most physiological chemistry laboratories the strengths of the reagents employed are very various, e.g. dilute acetic acid may be 1 per cent., or 2 per cent., or 5 per cent., or even 10 per cent. In order that all workers may employ a reagent of standard concentration, a list of reagents has been carefully drawn up and is appended.

COLOURED PLATE

ABSORPTION SPECTRA . . . . *Frontispiece*

(From Hoppe-Seyler Thierfelder's " Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse ")

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DEFINITION.

THE substances composing the organic material upon which the phenomena of life depend, and the great majority of the products of vital activity, are mixtures of compounds of the element Carbon.

From these substances the chemist has isolated numerous pure carbon compounds and prepared others; he has also synthesized carbon compounds from their elements.

Some 100,000 carbon compounds are now known. The possibility of their existence is due to the unique property which the element carbon possesses of being able to combine with itself; compounds are known which contain in their molecules from one to sixty atoms of carbon directly joined together.

Of these 100,000 carbon compounds only a small number are directly concerned in vital processes.

The chemistry of all the carbon compounds is termed organic chemistry.

The chemistry of those carbon compounds which are the constituents of living matter and are concerned in vital processes is physiological or biological chemistry. The changes which they undergo and the functions which they fulfil in the living plant or animal form the subject of chemical physiology.

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93

RECOGNITION OF AN ORGANIC COMPOUND.

Organic compounds are distinguished from inorganic compounds by being combustible, that is, when heated they will char, sometimes take fire, and will, on prolonged heating, completely burn away leaving no ash. Inorganic compounds when heated do not char and they leave a residue.

There are a few exceptions to this general rule, e.g. oxalic acid and its salts amongst the organic compounds, and the ammonium salts amongst the inorganic compounds. An oxalate will leave a residue of the oxide of the metal with which it is combined.

The following experiments exemplify these statements:—

1. Heat a small piece of paraffin wax upon platinum foil; it will melt, take fire, and will completely burn away leaving no residue.

2. Heat a crystal of cane sugar in the same way; it will melt, char, and on further heating will disappear completely.

3. Heat a few crystals of common salt on platinum foil; they will melt and unless heated very strongly, e.g. with a blowpipe flame, they will remain as a solid white mass when allowed to cool.

4. Heat a small piece of soap: as before, it will char, the vapours evolved may take fire, and when the charred particles have all vanished a white or nearly white residue will remain.

In this way substances composed of organic and inorganic matter can be recognized. The composition of the inorganic residue can only be found out by the usual methods of inorganic analysis when the organic matter has been destroyed by heating.

5. In the same way heat a little oxalic acid or an oxalate, e.g. calcium oxalate, and observe the results.

6. Also heat some ammonium chloride and note that it leaves no residue.

CONSTITUENTS OF A CELL.

A living cell consists of a mixture of organic and inorganic substances dissolved or suspended in water; water makes up about three quarters of the total mass of the cell, the other constituents about one quarter.

The twelve elements: carbon, hydrogen, nitrogen, oxygen, sulphur, phosphorus, chlorine (iodine), sodium, potassium, calcium, iron, and magnesium enter into the composition of the various compounds which compose the material of all living organisms; of these, only oxygen and nitrogen exist in the free state dissolved in the liquid.

The inorganic compounds are water and salts, chiefly the chlorides, sulphates, and phosphates of sodium, potassium, calcium, magnesium.

The organic compounds are very numerous in their variety. Proteins, carbohydrates, and fats are the essential substances, also the most complex. Alcohol, lactic acid, urea represent stages in the processes of their building up and breaking down, i.e. in their synthesis and decomposition or anabolism and catabolism.

DETECTION OF THE ELEMENTS IN A CELL.

1. Carbon, Hydrogen, Nitrogen.

Heat gently in a crucible about a gramme of dried yeast, previously powdered finely in a mortar. The mass chars, and volatile and combustible substances are evolved—presence of carbon and hydrogen. Continue the heating until these substances cease to be given off, and the mass no longer changes on further heating.

Notice the smell of burning flesh and hair—presence of nitrogen.

2. Sodium, Potassium, Chlorine, Phosphorus, Sulphur, etc.

Allow the mass to cool, extract it with warm water, filter and wash. Examine the filtrate A and the residue B.

Filtrate A.

The filtrate A contains those substances which are soluble in water and which are volatilized on heating to a higher temperature, e.g. sodium chloride.

Ascertain the reaction. It is generally alkaline owing to the presence of alkali carbonates and phosphates. Test separate portions for sulphuric acid, phosphoric acid, carbonic acid, hydrochloric acid, potassium, sodium; calcium sulphate is occasionally found in this extract, but rarely.

Residue B.

Heat the residue B still further until all the carbon has been oxidized away and a nearly white mass remains; this mass contains the substances insoluble in water, e.g. calcium phosphate.

Dissolve it in hydrochloric acid and note the presence or absence of carbonates by effervescence of carbon dioxide. Filter from any unburnt carbon and test the filtrate for phosphoric acid, calcium, magnesium, iron, as follows:—

Add ammonium chloride and ammonia: filter.

(*a*) The precipitate is white or yellowish-red in colour, and can consist of the phosphates of calcium, magnesium, iron. Dissolve a small portion in nitric acid and test for phosphoric acid with ammonium molybdate.

Treat the remainder with acetic acid. Boil for some time; yellowish-red flakes consisting of ferric phosphate remain undissolved; filter them off, dissolve in dilute hydrochloric acid and test for iron with potassium ferrocyanide or potassium thiocyanate.

$K_4 Fe CN_6 \cdot 3H_2O$

ferric ferrocyanide $\rightarrow$ $Fe_4(FeCN_6)_3$

Test the filtrate for calcium with ammonium oxalate, completely remove it by gently warming and filtering, and treat the filtrate with ammonia. If magnesium be present a crystalline precipitate will form either at once or after a short time.

(*b*) The filtrate may contain calcium and magnesium. Treat it with ammonium oxalate to remove calcium if present, and test for magnesium in the filtrate with sodium phosphate.

$Na_2 H PO_4 \boxed{12H_2O}$

Am molybdate + nitric acid

→ ammon oxide / readily [illegible]

LIFE-CYCLE OF THE ELEMENTS.

During the life processes of animals and plants an interchange of inorganic into organic and of organic into inorganic compounds occurs. The change of inorganic into organic is effected by the plant from the carbon dioxide in the atmosphere, ammonia and salts in the soil. The change of organic into inorganic is effected by the animal. The organic compounds synthesized by the plant enter the animal as food and are converted into carbon dioxide, which leaves the body by the lungs, salts, urea and other simple organic compounds, which leave the body in the urine. The fæces contains matter which has not been utilized by the animal. The urea and the other organic compounds in the excreta are finally broken down into carbon dioxide, ammonia, etc., by bacteria. There is thus a complete life-cycle of the elements.

DETECTION OF THE ELEMENTARY END PRODUCTS IN THE LIFE-CYCLE IN ANIMALS.

1. Carbon.

(*a*) On breathing into lime or baryta water, there is formed a large deposit of calcium or barium carbonate. Nearly all of the carbon is eliminated as carbon dioxide by the lungs; a small quantity only by the kidney.

(*b*) Evaporate down about 5 c.c. of urine in a porcelain basin to dryness over a small flame. Continue the heating when the residue is dry. It chars —presence of carbon.

2. Nitrogen.

Boil about 5 c.c. of urine in a small flask with caustic soda. Ammonia, due to the decomposition of the urea, is evolved, which may be recognized by its smell and the blueing of moistened red-litmus paper.

3. Chlorine.

To some urine add silver nitrate. A white precipitate of silver chloride and silver phosphate is formed. Acidify with nitric acid. This dissolves the silver phosphate, leaving the silver chloride, which is soluble in ammonia after filtering off. The silver phosphate is again precipitated if the filtrate be made alkaline with ammonia.

4. Phosphorus.

Phosphorus is present in urine as acid phosphates of the alkalies, and of calcium and magnesium.

(*a*) Render some urine alkaline with caustic soda, and heat gently. Calcium phosphate and magnesium phosphate are precipitated. Filter. To the filtrate add magnesia mixture. The soluble alkaline phosphates are precipitated as ammonio-magnesium phosphate (triple phosphate).

(*b*) Acidify some urine with acetic acid containing sodium acetate, and then add uranium acetate solution so long as there is a precipitate. Heat to 80° C. to complete the precipitation.

(*c*) Acidify with concentrated nitric acid, add excess of ammonium molybdate solution and heat to 50° C. A yellow coloration and then a precipitate of ammonium phosphomolybdate appears.

5. Sulphur.

Add baryta water to some urine, so long as a precipitate forms. Barium phosphate and barium sulphate are precipitated. Acidify with hydrochloric acid. The barium phosphate is dissolved, leaving the barium sulphate.

6. Sodium.

Evaporate 20 c.c. urine to dryness, and introduce some of the residue into a Bunsen flame by means of a platinum wire. Yellow colour. Spectroscope shows yellow sodium line.

7 Potassium.

Evaporate 100 c.c. urine to $\frac{1}{8}$ of its volume. Cool and filter. To filtrate add some concentrated tartaric acid solution, or sodium hydrogen tartrate. Mix well and allow to stand in cool place. Potassium hydrogen tartrate separates out, mixed with some ammonium hydrogen tartrate.

8. Calcium and Magnesium.

Render 200 c.c. urine alkaline with ammonia. After a few minutes filter off the precipitate of calcium and magnesium phosphates. Dissolve this precipitate in acetic acid, and filter if necessary.

To the clear filtrate add ammonium oxalate and heat gently. Calcium oxalate is precipitated. Filter.

Make the filtrate alkaline with ammonia. Ammonium magnesium phosphate forms on standing.

ISOLATION AND PREPARATION OF PURE ORGANIC COMPOUNDS.

CRITERIA OF THEIR PURITY.

The organic substances which compose all animal and vegetable cells consist of a mixture of numerous compounds. In order to investigate their evolution and their degradation in nature it is necessary to separate these compounds from one another and to prepare each of them in a state of purity. The chemical and physical properties of the pure substance can then be ascertained. Knowledge of the pure compounds shows their chemical relationship to one another and an idea of their rôle in nature is obtained. This idea is proved or disproved by an investigation of the changes which the organism, as a whole or individual portions of it, can effect in these substances.

In the study of the chemical properties of the compounds, other compounds are formed by their interaction. These compounds also require isolation and purification. The principal operations in organic and physiological chemistry will thus consist in the isolation and preparation of pure compounds.

Solid organic compounds are more numerous than liquid; gases are comparatively rare.

I. Solids.

(*a*) When two or more solids are present, only one may be soluble in a given solvent, e.g. a mixture of fat and protein may be separated by extracting with ether, which dissolves the fat leaving the protein. See under "Fats," p. 55.

(*b*) If the constituents of a mixture are all soluble in the same solvent they can be separated by fractional crystallization. On dissolving the mixture in a given solvent and evaporating the more insoluble compound separates out first when crystallization occurs, and is filtered off; the filtrate contains the more soluble compound, and this is obtained by evaporating until crystallization again occurs. The several crops of crystals require recrystallization from a suitable solvent, e.g. water, alcohol, benzene, to obtain a pure preparation.

(*c*) A mixture of solids in a solution may be separated by acidifying or making the solution alkaline, when precipitation may occur. In this case the substance precipitated will be either an acid or a base: thus benzoic acid and hippuric acid (pp. 132, 106) are precipitated when hydrochloric acid is added to a solution of their salts. Caseinogen (p. 177) is precipitated when milk is acidified with acetic acid. Aniline, quinine, strychnine are precipitated when caustic soda is added to their solution in acids.

These reactions depend upon the fact that the acid or base precipitated is insoluble or soluble only with difficulty in the acid or alkaline solution.

Acid substances may again be separated by precipitation as salts with heavy metals. Basic substances may be separated as salts with acids, e.g. urea nitrate, or as double salts with gold or platinum chloride, e.g. choline platinochloride.

(*d*) Two solids in a solution may be separated by extraction with a new solvent, which is not miscible with the first. The liquids are then separated, and the substances are obtained on evaporation of the two solvents, e.g. a mixture of succinic acid and urea in water is separated by extracting the solution with ether which dissolves the succinic acid leaving the urea in the water.

Lactic acid is usually separated in this way from an aqueous extract of muscle.

1. Separate Benzoic Acid from Solution and Purify it by Recrystallization.

To the given solution of sodium benzoate add a slight excess of dilute hydrochloric acid. Benzoic acid is precipitated. Filter off the precipitate and wash it free from hydrochloric acid with water.

The filtration of crystalline organic compounds is best effected by means of a perforated porcelain plate placed in a funnel or a complete funnel of porcelain of this pattern (Buchner), and suction as in the accompanying figure.

The perforations are covered over with a filter paper of the right size; this is wetted with the liquid and sucked down. The substance is then placed on the paper when the liquid is sucked off; it is then washed with water and pressed down with a spatula so that the wash water is drained off as completely as possible.

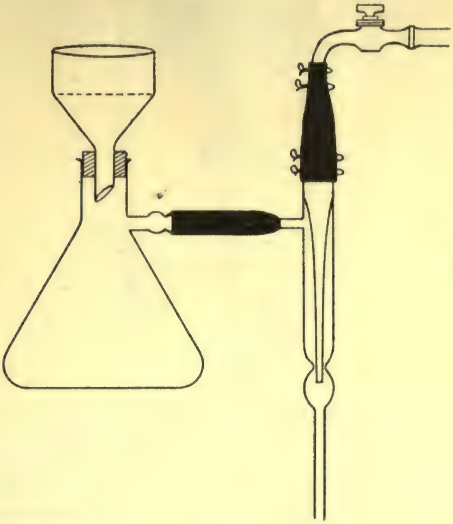


Fig. 1.

Then place the substance in a small flask or beaker and dissolve it in boiling water, sufficient only to just dissolve it being used. Whilst hot, filter the solution through a folded or pleated filter paper into a clean beaker, cover the beaker with a clock glass or a piece of clean paper and allow to cool. Pure benzoic acid will crystallize out. Filter off the crystals, wash them with a little fresh solvent and dry the crystals thoroughly between sheets of filter paper. Determine its melting-point (p. 11).

2. Separate Quinine from Solution.

To the solution of quinine hydrochloride add a slight excess of sodium carbonate solution. The quinine is precipitated. Shake the solution with ether, allow the liquids to separate and then decant off the upper ethereal layer on to a watch glass. Crystals of quinine will remain on allowing the ether to evaporate.

3. Separate and Purify the Mixture of Succinic Acid and Urea.

Place the aqueous solution of succinic acid and urea in a separating funnel and add about a quarter its volume of ether. Shake up the two solvents.

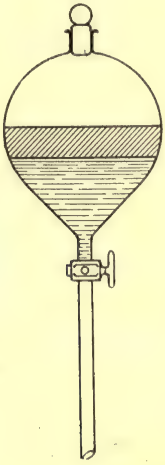


FIG. 2.

An increase in pressure usually occurs when ether and water and air are first shaken together in a separating funnel. Whilst shaking the tap of the funnel must therefore be occasionally opened after allowing the liquids to settle at the other end. After thoroughly shaking allow the liquids to separate from one another, remove the stopper from the funnel and run out the heavier liquid (water) into a clean beaker. Then pour out the ether layer into a clean basin and allow it to evaporate.

Extract the aqueous layer two or three times with fresh quantities of ether. Combine the ethereal extracts and allow them to evaporate. Succinic acid will be obtained. Recrystallize it from hot water and determine its melting-point.

Evaporate the aqueous solution on the water bath: urea remains. Recrystallize it from hot alcohol heated on the water bath. Determine its melting-point.

Determination of the Melting-point.

A pure solid organic compound melts sharply at a definite temperature. An impure organic compound does not melt sharply and it melts at too low a temperature. The melting-point of a compound also helps in its identification.

Introduce a small quantity, sufficient to occupy a length of about 1 cm., of the finely powdered substance into a melting-point tube. This consists of a thin-walled glass tube about 1 mm. in diameter and 5-6 cm. long and closed at one end. It is made by heating a dry piece of glass tubing in a blow-pipe flame and when soft drawing it out; the long capillary tube thus made is cut into the required lengths and one end is sealed by holding it in the flame.

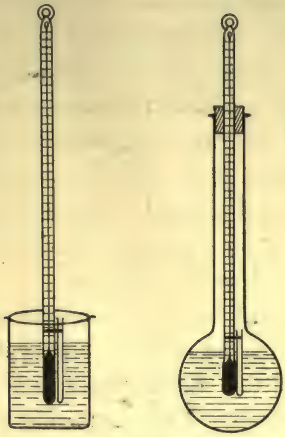


FIG. 3.

Attach the melting-point tube when filled by a narrow rubber band to a thermometer so that the substance is on a level with the mercury of the thermometer. Suspend this by means of a clamp in a beaker of concentrated sulphuric acid which is placed upon a wire gauze in such a position that it may be gently heated by a flame. Gradually heat the beaker and at the same time stir the liquid with a glass stirrer. Note the temperature at which the substance melts.

unsafe

Instead of a beaker a small flask with long neck may be employed to contain the sulphuric acid; the thermometer is then secured in a cork into which a notch is cut so as to see the graduations of the thermometer and to allow the air to escape when the flask is heated. The melting-point tube in this case may be attached to the thermometer by adhesion.

Take the melting-point of the recrystallized benzoic acid, succinic acid, and urea. Benzoic acid melts at 121° C., succinic acid melts at 185° C., urea melts at 132° C.

II. Solid and Liquid.

Obviously the procedure to adopt is filtration.

The solid may, however, be soluble in the liquid; the liquid will therefore contain dissolved solid. Separation may be effected by distilling off and collecting the liquid when the solid remains, or by extracting with a solvent which dissolves only one of the substances. Each is purified according to the methods for solids and liquids.

III. Liquids.

A. Separation.

Two liquids, if they are not miscible, are easily separated from one another. They are placed in a tap-funnel or separating-funnel and the heavier liquid run out. (Compare p. 10.)

A mixture of two or more miscible liquids may be separated from one another by fractional distillation (hydrocarbons, alcohol and water).

Two liquids, if they are miscible, may not both be soluble in the same solvent; this solvent must not be miscible with the other constituent, e.g. chloroform and alcohol. Alcohol is soluble in water, but chloroform is not; hence on shaking with water, the chloroform will sink under the alcohol and water mixture.

Though chloroform and water are apparently not miscible, each exerts a solvent action on the other. The water will contain a little dissolved chloroform and the chloroform a little dissolved water. The water is removed by "drying" the chloroform. This is effected by placing lumps of calcium chloride in the liquid and allowing to stand for 12-24 hours (p. 13).

B. Purification.

Liquids are purified by distillation. A pure liquid has a constant boiling-point.

IV. Liquid and Gas.

Gases are soluble in liquids. The gas may be obtained by heating the solution and collecting the gas over a liquid in which it is not soluble. The liquid remains behind. Each is then purified according to the methods for liquids and gases.

V. Gases.

A mixture of gases may be separated by treating with suitable solvents which dissolve one or several of the constituents. Gases are best separated by condensation by cold to liquids followed by fractional distillation.

Gases are most easily purified by passing through substances, such as water, sulphuric acid, etc., which dissolve the impurity but not the gas.

Separate Chloroform from Alcohol and Purify the Chloroform by Distillation.

Mix together 50 c.c. each of chloroform and alcohol in a small tap-funnel and then add 100 c.c. of water and shake up thoroughly. Notice the lower layer of chloroform; run this out into a clean flask.

Dry the separated chloroform with calcium chloride (p. 12), filter and distil (below).

Distillation of a Liquid and Determination of its Boiling-point.

Place the 50 c.c. of dry chloroform in a small dry clean distilling flask (a round-bottom flask with side tube in its neck) filling it only about half or at most two-thirds. Close the neck with a cork which carries a thermometer, and adjust it so that the bulb of the thermometer is just below the opening of the side tube and not touching the walls. Connect the side tube by means of a cork with a clean dry condenser, and place a receiver (flask) at the other end of the condenser.

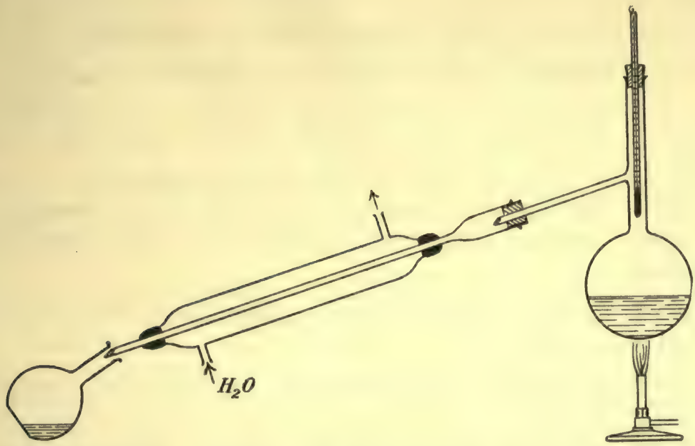


FIG. 4.

Gently boil the liquid in the distilling flask placed on a water bath and observe the thermometer. The mercury column rises rapidly at first and then becomes stationary at a definite temperature. *This is the boiling-point of the liquid.* Continue the heating until all the liquid boiling at this temperature has distilled over into the receiver. The liquid in the receiver will be pure chloroform; the portion remaining in the distilling flask contains the impurities.

COMPOSITION OF ORGANIC COMPOUNDS.

A pure carbon compound can now be analyzed, i.e. what elements besides carbon are contained in it, and in what proportions.

The carbon compounds are compounds of carbon with the elements hydrogen, oxygen, nitrogen, sulphur, phosphorus, the halogens, etc.

These elements may be in combination with the carbon either one at a time, two at a time, three at a time, etc. Thus we may have:—

Carbon combined with oxygen only, e.g. CO, CO_2. C_nH_{2n+2}

Carbon combined with hydrogen only, e.g. the hydrocarbons, benzene. [illegible]

Carbon combined with nitrogen only, e.g. cyanogen. CN_2

Carbon combined with hydrogen and oxygen, e.g. alcohol, ether, $(C_2H_5)_2$ etc. $C_nH_{2n+2}O$

Carbon combined with hydrogen and nitrogen, e.g. hydrogen cyanide, the nitriles, the amines such as trimethylamine.

Carbon combined with hydrogen and sulphur, e.g. mercaptan. [illegible]

Carbon combined with hydrogen and chlorine, e.g. ethyl chloride, chloroform. – [illegible] – $CHCl_3$

Carbon combined with hydrogen, oxygen, and nitrogen, e.g. urea, uric acid, the amino acids.

Carbon combined with hydrogen, oxygen, and sulphur, e.g. sulphonal.

Carbon combined with hydrogen, oxygen, nitrogen, sulphur, e.g. the proteins; in these compounds phosphorus may also be present and they belong to the most complex of the organic compounds.

A. ELEMENTARY COMPOSITION.

DETECTION OF THE ELEMENTS.

1. Carbon and Hydrogen.

(*a*) Heat gently a small portion of the substance provided (cane sugar) in a test tube. Observe the charring—presence of carbon; and the condensation of water on the sides of the tube where it is cool—presence of hydrogen.

(*b*) Dry thoroughly about 5 grms. of finely powdered cupric oxide by heating it in a small crucible. Whilst still warm [1] mix it with a little oxalic acid and introduce the mixture into a hard glass tube. Close the end with a cork through which passes a glass tube, bent at right angles, and dip this into a little baryta water contained in a small beaker or test tube. Heat the mixture in the hard glass tube. Water will condense on the cooler parts of the tube—presence of hydrogen; and the baryta water will become turbid owing to the formation of barium carbonate—presence of carbon.

$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 + H_2O$

2. Nitrogen.

(*a*) **As Ammonia.** Grind up a portion of the substance (caseinogen) with soda-lime. Heat in a dry test tube and observe by smell and by litmus paper that ammonia is given off. White fumes are produced when a glass rod dipped in hydrochloric acid is held over the mouth of the tube. (Will and Varrentrapp's method.)

The peculiar smell of burning flesh, horn, etc., produced on heating such substances as in (1), also indicates the presence of nitrogen. Heat a portion of the caseinogen and observe this smell.

(*b*) **As Sodium Cyanide.** Heat a small piece of metallic sodium in a dry test tube of hard glass until the metal begins to boil, then add in successive minute portions a small quantity of the substance (dried egg albumin or urea). Continue heating a short time, then cool and break the lower end of the tube in a mortar, and add a little alcohol; when effervescence has ceased add water, warm and filter. To the filtrate add some ferrous sulphate solution (this must be freshly prepared by dissolving a few small crystals in a little water) and caustic soda and boil for a few minutes. Cool, add a drop or two of ferric chloride and excess of dilute hydrochloric acid. A precipitate or coloration of Prussian blue indicates the presence of nitrogen (Lassaigne's method).

Note.—It is important that the substance be made to come into proper contact with the sodium.

Castellana's Modification of this Test.—Mix intimately a small quantity of the substance with about ten times its quantity of equal parts of magnesium powder and dry sodium carbonate, and gently heat until the magnesium burns, then heat to redness as with the sodium (*b*). Carry out remainder of process as described above, i.e. breaking tube in water, etc.

---

[1] As cupric oxide takes up water on cooling it must be used warm, otherwise it must be allowed to cool in a desiccator over sulphuric acid.

Look up

3. Halogens.

(*a*) **Beilstein's test.** A piece of copper wire is heated in a Bunsen flame until the flame is no longer coloured green. A little of the substance, e.g. chloroform, is placed on it and it is again heated. Copper chloride is formed which colours the flame green.

omit

(*b*) **Halogen** may also be detected by means of sodium employed just as in the nitrogen test. The filtered solution is acidified with nitric acid, boiled to remove any hydrocyanic acid which will be formed if the substance also contains nitrogen, and then treated with silver nitrate.

The nature of the halogen may be determined by treating a little of the acidified solution with chlorine water, and then testing with starch solution for iodine, or by extracting with carbon bisulphide or chloroform for bromine.

$CaO + Na_2O$

(*c*) **Heating with Lime.** Halogens are best detected by heating with quicklime. The substance is finely powdered and mixed intimately with lime (if liquid, e.g. chloroform, the lime is moistened with the substance) and then heated strongly. When cool, water is added and the lime dissolved in nitric acid. On adding silver nitrate a precipitate of silver halide is obtained if halogen be present.

Chloride

omit

(*d*) **Detection of Iodine in the Thyroid Gland.** For this purpose a special method is used owing to the small quantity of iodine present.

Cover a tabloid of thyroid gland extract with water in a crucible. Then add twice its amount of solid caustic soda, evaporate down and heat until the mass is thoroughly charred. Remove the flame, add carefully about an equal quantity of powdered potassium nitrate until the mass burns; apply heat, if necessary. Cool, dissolve the mass in hot water and filter. Acidify the filtrate, when cold, with sulphuric acid, add a few c.c. of chloroform and shake. A violet coloration of the chloroform indicates iodine.

4. Sulphur.

omit

(*a*) As sodium sulphide. Heat a small portion of the substance with metallic sodium as described under 2 (*b*). Break the hot tube in a little water, filter and test for sodium sulphide with (1) lead acetate, (2) sodium nitroprusside.

[illegible]

(*b*) As sulphate. Fuse a small portion of the substance provided (dried fibrin) in a crucible with three times its quantity of fusion mixture $\{2\ KNO_3 + Na_2CO_3\}$. Heat cautiously at first round the edge and continue heating after the fusion until all charred particles have vanished. Cool, extract with hot water and test the filtered solution for sulphates with barium chloride in the presence of mineral acid (HCl or HNO_3).

Na_2SO_4
$BaSO_4$.

[illegible] for phos. [illegible]

S is fused with soda carbonate and oxidised by pot. nitrate.

5. Phosphorus.

(*a*) Fuse, as described for sulphur, some caseinogen with fusion mixture. Extract the fused mass with hot water and divide the solution into two parts. To the one part add excess of nitric acid and ammonium molybdate and warm; a yellow precipitate indicates phosphoric acid; to the other part add excess of ammonia and test for phosphates with magnesia mixture.

NH_4 P Mg

(*b*) By Neumann's method. Cover a small quantity of caseinogen in a small flask with concentrated sulphuric acid and an equal volume of concentrated nitric acid. Heat gently over a small flame (in the draught chamber) until the mixture becomes colourless. If it becomes brown, cool, add more nitric acid and heat again. When colourless, cool, add water and a little ammonium nitrate solution and heat nearly to boiling; then add some ammonium molybdate solution. A yellow colour or precipitate indicates the presence of phosphoric acid.

6. Other Elements.

Amongst natural compounds the most important other elements combined with carbon are iron, e.g. iron in hæmoglobin, ferrocyanides, and magnesium, e.g. magnesium in chlorophyll. Copper is found in certain other animal pigments. Silicon in present in certain vegetables, e.g. in grasses. Organic silicon, arsenic, and antimony compounds have been prepared in the laboratory. These elements are best detected after the organic matter has been completely removed by burning either alone or in the presence of an oxidizing agent (fusion mixture). Thus :—

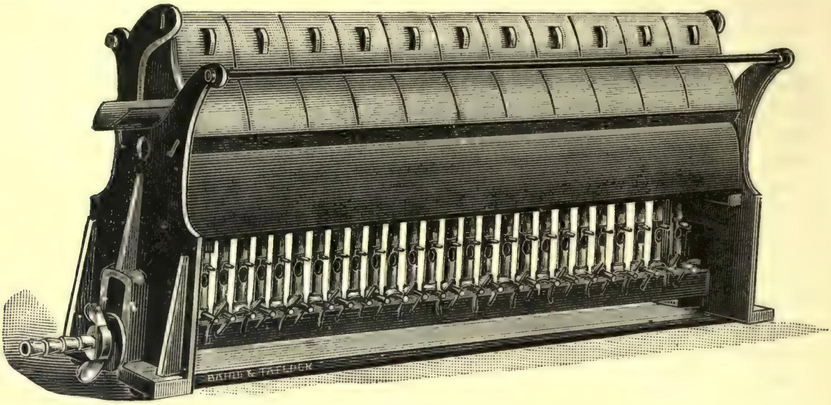
Detection of Iron in Hæmoglobin. Heat a small portion of hæmoglobin in a crucible with 3-4 times its quantity of fusion mixture until all the organic matter has been oxidized. Cool, dissolve the mass in dilute hydrochloric acid, filter, and test the filtrate with (*a*) ammonium thiocyanate, and (*b*) potassium ferrocyanide. → $Fe_4(FeCN_6)_3$
ferric ferro cyanide.

B. QUANTITATIVE COMPOSITION.

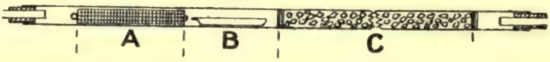
1. Carbon and Hydrogen.

As previously seen, an organic compound when oxidized with copper oxide is converted into carbon dioxide and water. The amount of each element contained in the compound is therefore determined by weighing the carbon dioxide and water produced from a known weight of the compound.

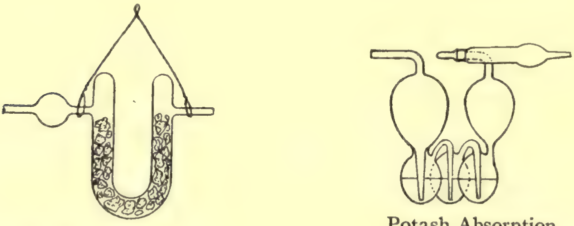
In practice, the analysis is carried out in a long combustion tube of hard glass, which is heated in a furnace. The tube is filled to two-thirds of its length with coarse copper oxide, a small boat (copper or porcelain) of suitable size, containing a known weight of the substance, is introduced and then a roll of oxidized copper gauze. This latter end of the tube is connected with a gasometer containing air (or oxygen) from which a current of air, freed from carbon dioxide and water by passing through potash and sulphuric acid or calcium chloride, is passed through the combustion tube so as to drive out the products of the combustion and to help in the oxidation. To the other end of the combustion tube are attached absorption tubes, of which there are various forms, to collect the carbon dioxide and water. The first absorption tube, generally of U-shape, contains calcium chloride or pumice wetted with concentrated sulphuric acid; the second, generally complex in shape so as to give several surfaces, contains caustic potash of 33 per cent. strength. These tubes are weighed before and after the combustion and their increase in weight gives the required data.



Combustion Furnace.



(From Price and Twiss' "Practical Organic Chemistry".)



Calcium Chloride Tube.

Potash Absorption Bulbs.

(From Price and Twiss' "Practical Organic Chemistry".)

FIG. 5.

It should be mentioned that the portion of the tube containing the coarse copper oxide is heated to the required temperature before the introduction of the boat; the roll of copper gauze is then heated and the heat is gradually extended from this point towards the boat and the copper oxide. All the carbon dioxide and water vapour previously contained in the combustion tube are expelled by passage of the air from the gasometer before the oxidation is commenced.

2. Nitrogen.

(*a*) **Dumas' Method.** When an organic compound containing nitrogen is heated with copper oxide, the whole of the nitrogen is given off as nitrogen. The gas given off from a known weight of substance is therefore collected and its volume measured, from which value its weight can be calculated.

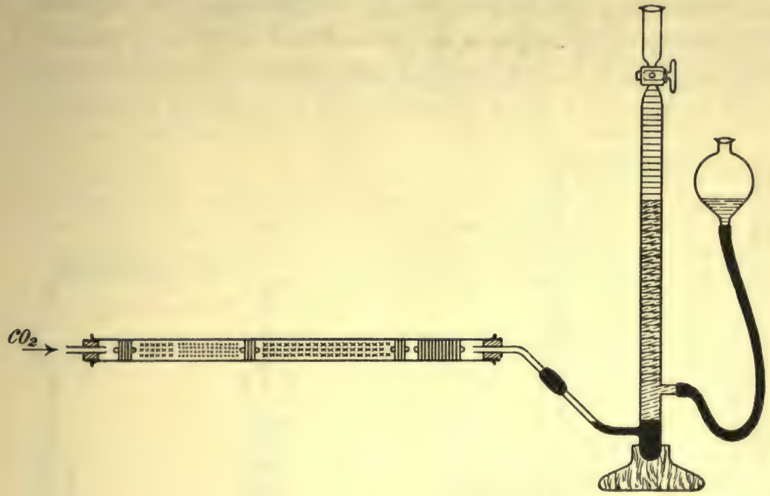


Fig. 6.

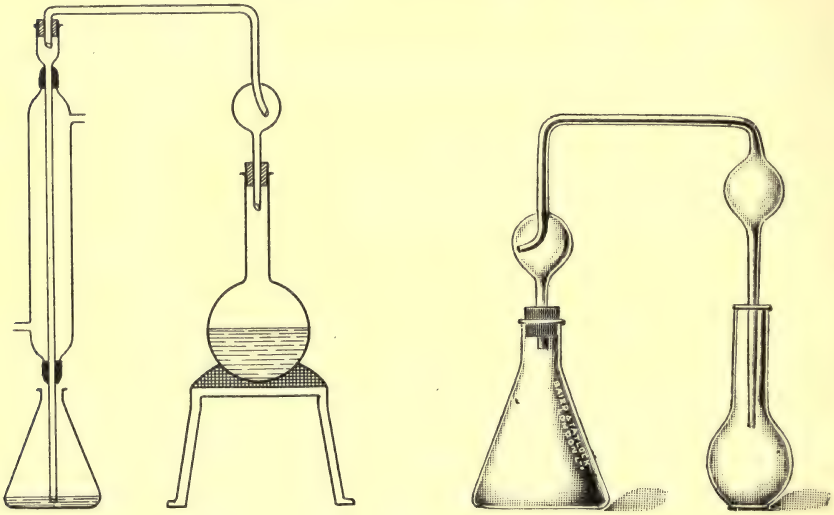
The analysis in practice is usually carried out in a similar way to that described for carbon and hydrogen. In this case, however, the substance is mixed with finely powdered copper oxide and introduced into the tube and a roll of reduced copper gauze is placed at the end of the combustion tube so as to reduce any oxides of nitrogen which may be formed to nitrogen. Also, instead of a current of air, a current of carbon dioxide is passed through the tube. The gas is collected in a Schiff's nitrometer over caustic potash which absorbs the carbon dioxide leaving the nitrogen.

There are several ways of passing the carbon dioxide through the combustion tube; it may be evolved from a Kipp apparatus or it may be evolved by heating magnesite either contained in a special tube or in the combustion tube which in this case is sealed at the end. It is obvious that before carrying out the analysis all the air must be expelled from the apparatus.

(*b*) **Kjeldahl's Method.** The principle of this method consists in oxidizing the substance with concentrated sulphuric acid; the nitrogen is converted into ammonia. The solution is then made alkaline with caustic soda and distilled. The ammonia is evolved and is collected in excess of standard acid; on subsequent titration with standard alkali the amount evolved is given by difference.

This method is much simpler to carry out than the Dumas' method, but it cannot be employed for all nitrogen-containing compounds; certain of them e.g. (osazones) give off a mixture of nitrogen and ammonia when oxidized with sulphuric acid; a portion of the nitrogen is therefore lost in the analysis.

On account of its simplicity it has found extensive use in physiological chemistry: the large group of compounds—the proteins—all contain nitrogen; the amount of protein in a solution is estimated by determining the nitrogen content (see below); and the amount of nitrogen in urine is a factor of importance in studying metabolism.



No. 1. Fig. 7. No. 2.

Estimation of Nitrogen in Egg-white Solution.

Measure out 5 c.c. egg white solution with a pipette into a clean round-bottomed Jena glass flask of 700 c.c. capacity. Add 10 c.c. of pure concentrated sulphuric acid, and also a small crystal of copper sulphate which helps in the oxidation. (Potassium sulphate is also sometimes added for this purpose.) Heat the flask over a gauze in a fume-cupboard until the liquid which at first becomes brown from charring becomes quite or nearly colourless, a process which takes about an hour. Allow to cool and then half fill the flask with distilled water. By means of a special distillation tube connect the flask to a condenser set up in a vertical position as in Fig. 7, No. 1.

Measure out with a pipette a quantity of standard sulphuric acid (5 c.c. $\frac{N}{1}$ or 50 c.c. $\frac{N}{10}$ H_2SO_4) into a clean flask (preferably conical) of about 600 c.c. capacity and place this below the condenser so that the end of the condenser just reaches the surface of the liquid.

Sometimes the distillation is carried out without using a condenser (Fig. 7, No. 2); the end of the special distilling tube is then dipped into the standard acid. There is in this case usually greater danger of the liquid being sucked back into the flask which is being distilled and further the glass is attacked by the ammonia with liberation of alkali, which causes inaccuracies in the determination.

Now remove the round-bottomed flask, add a piece of porous earthenware and run in under the acid excess of caustic soda solution (50 c.c. 40 per cent. for every 10 c.c. concentrated H_2SO_4 used) and connect again to the condenser seeing that all corks fit tightly. Mix the soda and acid and distil off the ammonia. In about half an hour the ammonia will have completely passed over into the standard acid. Lower the flask and allow the water to wash out the inside of the condenser tube for about two minutes: at the same time test the distillate with litmus to ascertain if any more ammonia is being evolved. Then wash the outside of condenser tube with distilled water and titrate the contents of the flask with $\frac{N}{10}$ alkali using methyl orange or alizarin red as indicator. It is preferable to add a few drops of indicator before the distillation is commenced in case more acid is required than that originally taken; the change in colour of the indicator gives notice of this fact.

The difference between the amount of standard alkali and standard acid gives the amount of ammonia evolved, from which the amount of nitrogen can be calculated:—

$$5 \text{ c.c. } \frac{N}{1}\ H_2SO_4 = 50 \text{ c.c. } \frac{N}{10}\ H_2SO_4.$$

$$\frac{37{\cdot}4 \text{ c.c. } \frac{N}{10} \text{ NaOH used.}}{12{\cdot}6 \text{ c.c. } \frac{N}{10} \text{ difference.}}$$

$$12{\cdot}6 \text{ c.c. } \frac{N}{10}\ H_2SO_4 = 12{\cdot}6 \text{ c.c. } \frac{N}{10}\ NH_3.$$

$$= 12{\cdot}6 \text{ c.c. } \frac{N}{10}\ \text{N}.$$

$$= 12{\cdot}6 \times 0{\cdot}0014 \text{ gm. N.}$$

∴ 5 c.c. solution contain 0·01964 „ N.

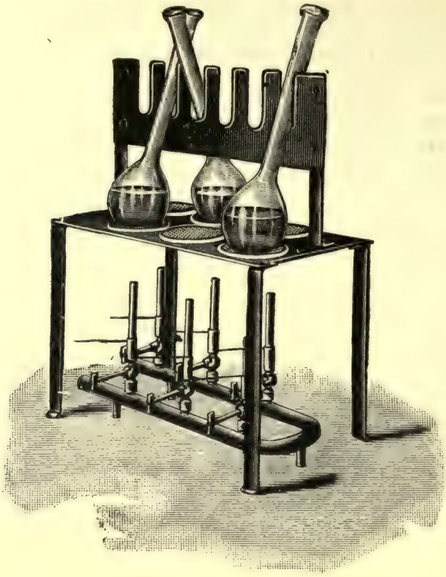
∴ 100 c.c. „ „ 0·3528 „ N.

The amount of protein to which this amount of nitrogen corresponds is ascertained as follows:—

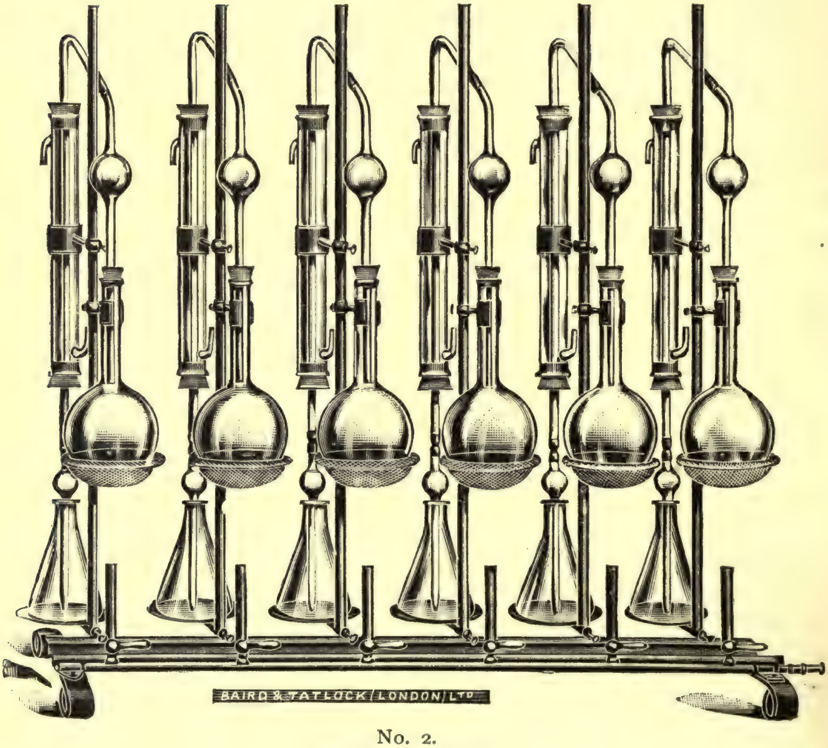
Proteins contain from 15-16 per cent. N. Taking 15·5 as the average value, $\frac{100}{15\cdot5}$ or 6·45 times the amount of nitrogen gives the amount of protein. The factor 6·25 is however generally used.

100 c.c. solution thus contain 6·25 × 0·3528 gm. protein = 2·2 gm.

In most laboratories there is an apparatus in which 6 determinations can be carried out at the same time as in Fig. 8.



No. 1.



No. 2.
Fig. 8.—Kjeldahl.

3. Halogens.

After completely oxidizing all the organic matter to carbon dioxide and water, the halogens are present as inorganic compounds and are estimated in the usual way.

In practice the commonest method is to oxidize the substance in a sealed tube with fuming nitric acid at 200° C. for several hours, a few crystals of silver nitrate being at the same time placed in the tube: silver halide is formed and this is washed out of the tube and weighed. (Carius.) Less frequently, halogens are estimated by heating the substance in a combustion tube with quicklime. The contents of the tube after the oxidation are dissolved in nitric acid, and the halogen precipitated with silver nitrate. The silver halide is filtered off, washed, dried, and weighed.

A convenient method of estimating halides is that described by Neumann. The substance is oxidized with a mixture of nitric acid and sulphuric acid. Hydrochloric acid, etc., is evolved and this is collected in a solution of silver nitrate. After boiling the solution for about half an hour to remove hydrogen cyanide which is also formed, the silver halide is filtered off, washed, dried, and weighed. (See "J. Physiol.," vol. **31**, p. 65.)

4. Sulphur.

This element may be estimated by the same method as the halogens (Carius).

It is more convenient to oxidize the substance in an open crucible with sodium or barium peroxide; then acidify with hydrochloric acid and weigh the barium sulphate formed. This is the usual method for estimating sulphur in urine.

5. Phosphorus.

Phosphorus is usually estimated in the same way as sulphur by the Carius method, the phosphoric acid formed being precipitated as ammonium magnesium phosphate.

The most rapid and convenient method is that of Neumann (cf. p. 17). The substance is oxidized in an open flask with a mixture of nitric and sulphuric acids. The phosphoric acid formed is precipitated as ammonium phosphomolybdate and this is then estimated by solution in excess of $\frac{N}{2}$ caustic soda and subsequent titration with $\frac{N}{2}$ sulphuric acid. The difference between $\frac{N}{2}$ NaOH and $\frac{N}{2}$ H_2SO_4 multiplied by 1·268 gives the number of milligrams of P_2O_5 in the given weight of substance taken. (See "J. Physiol.," 1906, **33**, 439.)

CALCULATION OF RESULTS.

With the exception of oxygen all the elements present in an organic compound are thus estimated. The amount of oxygen is found by difference.

From the figures obtained the percentage composition is calculated, i.e. the amount given by 100 grammes of substance, thus :—

0·2009 gm. substance gave 0·2987 gm. CO_2 and 0·1092 gm. H_2O.
0·1887 gm. „ „ 15·2 c.c. moist N at 16·5° and 767 mm.

$$\text{Now, } 0{\cdot}2987 \text{ gm. } CO_2 = 0{\cdot}2987 \times \frac{12}{44} \text{ gm. C} = 0{\cdot}2987 \times \frac{3}{11} \text{ gm. C} = 0{\cdot}0815 \text{ gm. C.}$$

$$0{\cdot}1092 \text{ gm. } H_2O = 0{\cdot}1092 \times \frac{2}{18} \text{ gm. H} = 0{\cdot}1092 \times \frac{1}{9} \text{ gm. H} = 0{\cdot}01213 \text{ gm H.}$$

$$\begin{aligned} 15{\cdot}2 \text{ c.c. moist N at 767 mm. and } 16{\cdot}5^\circ \text{ C.} &= \frac{15{\cdot}2 \times 753^* \times 273}{760 \times 289{\cdot}5} \text{ c.c. at } 0^\circ \text{ and 760 mm.} \\ &= 14{\cdot}2 \text{ c.c.} \\ &= 14{\cdot}2 \times \frac{28}{22{,}400} \text{ gm.} \\ &= 0{\cdot}01775 \text{ gm. N.} \end{aligned}$$

$$\begin{aligned} \therefore \text{ percentage of C} &= \frac{0{\cdot}0815 \times 100}{0{\cdot}2009} = 40{\cdot}56. \\ \text{H} &= \frac{0{\cdot}01213 \times 100}{0{\cdot}2009} = 6{\cdot}04. \\ \text{N} &= \frac{0{\cdot}01775 \times 100}{0{\cdot}1887} = 9{\cdot}40. \\ \text{O by difference} &= \underline{44{\cdot}00.} \\ \text{Total} &= 100{\cdot}00 \end{aligned}$$

The formula is then obtained by dividing the percentages by the atomic weights of the elements; the ratio of the number of atoms to each other is then obtained by dividing by the lowest value :—

$$\begin{aligned} &\text{C} \quad \frac{40{\cdot}56}{12} = 3{\cdot}38 \div 0{\cdot}67 = 5. \\ &\text{H} \quad \frac{6{\cdot}04}{1} = 6{\cdot}04 \div 0{\cdot}67 = 9. \\ &\text{N} \quad \frac{9{\cdot}40}{14} = 0{\cdot}67 \div 0{\cdot}67 = 1. \\ &\text{O} \quad \frac{44{\cdot}0}{16} = 2{\cdot}75 \div 0{\cdot}67 = 4{\cdot}1. \end{aligned}$$

The formula of the compound is therefore $C_5H_9NO_4$.

In any estimation only a difference of 0·2-0·3 per cent. is allowed between the values found and those calculated from the formula. The calculated values are :—

C = 40·81. diff. = − 0·25.
H = 6·12. = + 0·08.
N = 9·52. = − 0·12.

The analysis was therefore sufficiently accurate.

---

* Vapour pressure of water at 16·5° C. = 14·0 mm. ∴ pressure on gas = 767 − 14 = 753 mm.

DETERMINATION OF THE MOLECULAR WEIGHT.

As will be seen later several organic compounds can have the same empirical formula, thus, for instance, lactic acid $C_3H_6O_3$ and glucose $C_6H_{12}O_6$ have the same empirical formula, namely CH_2O.

In order to ascertain which of these formulæ is the correct one, a molecular weight determination is carried out, i.e. the weight of the molecule of the substance compared with that of an atom of hydrogen (Avogadro's law).

The methods employed to determine the molecular weight are of two kinds, (*a*) physical, (*b*) chemical.

(*a*) Physical Methods.

1. **Victor Meyer's Method.** Of the physical methods, that by Victor Meyer is the most frequently used when the substance can be vaporized without decomposition. A known weight of the substance is converted into vapour at a temperature 40-50° above its boiling-point in a special apparatus. The air previously contained in the apparatus is displaced by the vapour, collected in a graduated cylinder and its volume measured; this volume, after making corrections for temperature and pressure, corresponds to that occupied by the substance.

Thus, if v c.c. are given by w grammes substance.

$$\therefore 22{,}400 \text{ c.c. are given by } \frac{w \times 22{,}400}{v}$$

$$= \text{mol. wt.}$$

The apparatus employed is shown in the accompanying figure. A liquid, boiling 40-50° above the temperature at which the substance is volatilized, is boiled in the round bulb of the outer vessel. As soon as the temperature is constant and no more air escapes from the inner vessel by the side tube, the inverted graduated cylinder, filled with water, is placed over the end of the side tube, the cork is removed and a known weight of substance, contained in a small glass vessel is dropped through the opening into the inner vessel and the cork is rapidly replaced. The vapour of the substance displaces an equal volume of air in the vessel, which is driven out and collected and measured in the graduated cylinder.

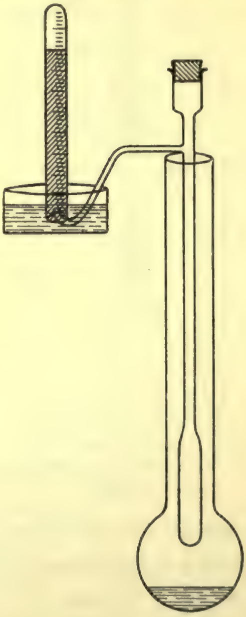


FIG. 9.

2. **Raoult-Beckmann Method.** When substances are dissolved in a liquid its freezing-point is lowered. It was shown by Raoult that the freezing-point was lowered the same number of degrees when weights of different substances proportional to their molecular weights were dissolved in the liquid. Each liquid was found to have a definite freezing-point. By employing this value as a constant, the molecular weight of an unknown substance can be found. It is given by the formula

$$M = \frac{100 \times C \times w}{dW},$$

where C is the constant, *w* the weight of the substance, W the weight of the solvent, and *d* the depression of the freezing-point.

Conversely by determining the lowering of the freezing-point, the amount of salt in a solution can be ascertained, e.g. in serum, urine.

Similarly, a rise in the boiling-point of a solvent, when substances are dissolved in it, will give the molecular weight of the substance.

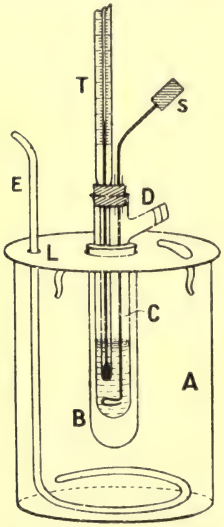


Fig. 10.—(From Findlay's "Practical Physical Chemistry")

The apparatus devised by Beckmann consists of the freezing-point tube C with side opening D. It is closed by a cork through which a Beckmann thermometer and a stirrer S (through a glass tube) pass. The Beckmann thermometer is a large thermometer graduated usually in $\frac{1}{1000}$ parts of a degree and having a range of only 5-6 degrees. The tube C is placed in a wider tube B which serves as a jacket and prevents too rapid cooling. This is fixed in position in the freezing mixture in the vessel A by a cork which fits the opening in the brass lid L. The brass lid has also openings for the passage of a stirrer E and a thermometer. In carrying out a determination a known weight of solvent is placed in C and its freezing-point is taken. The tube is then removed and the solid allowed to melt. A known weight of substance is then introduced through D and the freezing-point again determined.

(*b*) Chemical Methods.

When the substance is an acid or a base the molecular weight can be determined by chemical methods.

In the case of an acid, the number of hydrogen atoms replaceable by a metal is ascertained by estimating the amount of metal. It is most usual to employ the silver salt of an acid. A known weight of salt is simply heated in a crucible and the metallic silver obtained is weighed.

The zinc salt or barium salt are also sometimes employed; a known weight of salt is heated with a drop of concentrated sulphuric in a crucible when zinc or barium sulphate is obtained from which the amount of barium or zinc is calculated, e.g.

a gm. of silver salt gave b gm. of silver.

$\therefore a - b$ will be the weight of organic matter combined with b gm. silver,

$\therefore \frac{(a-b)}{b} \times 107{\cdot}9$ " " " " $107{\cdot}9$ gm. silver.

This amount of silver corresponds to 1 gm. of hydrogen replaced by the metal.

$$\therefore \text{molecular weight is } \frac{(a-b)}{b} \times 107{\cdot}9 + 1.$$

In the case of a base, the formation of double salts with metallic salts, such as platinum chloride, mercuric chloride, and the estimation of the amount of metal in a known weight of the compound gives the molecular weight. Ammonia and platinum chloride give the compound ammonio-platinum chloride.

$$(NH_3 . HCl)_2 . PtCl_4.$$

The organic bases form analogous compounds, the base replacing the ammonia. Their general formula is therefore

$$(B . HCl)_2 . PtCl_4 \text{ or } B_2 . H_2PtCl_6.$$

The molecular weight of 2 molecules of base is thus

$$B_2 . H_2PtCl_6 - H_2PtCl_6.$$

e.g. x grammes of salt gave y grammes of platinum.

$$\therefore 194{\cdot}8 \text{ gm. platinum will be given by } \frac{194{\cdot}8 \times x}{y} \text{ gm. salt } B_2 . H_2PtCl_6.$$

Now $194{\cdot}8$ gm. platinum are contained in $409{\cdot}8$ gm. H_2PtCl_6.

$$\therefore \frac{194{\cdot}8 \times x}{y} - 409{\cdot}8 \text{ is the molecular weight of } B_2.$$

$$\text{Hence molecular weight of 1 molecule of base is } \frac{\frac{194{\cdot}8 \times x}{y} - 409{\cdot}8}{2}$$

IDENTIFICATION OF AN ORGANIC COMPOUND.

Knowing now the formula of a pure organic compound from its analysis and molecular weight, the compound must be identified. It may be a known compound or an unknown compound.

To ascertain if the compound is known reference is made to Richter's "Lexicon of Carbon Compounds"[1] in which the melting-points and other constants of the various compounds is given. Corresponding properties identify the substance.

If the compound be unknown, further analysis is necessary; it must be ascertained to what group of carbon compounds the unknown body belongs, whether it is e.g. an alcohol, an ester, an acid, a carbohydrate, an amide, an amine, a protein, etc. With the complex natural substances this is a matter of great difficulty, and it may take many years before a question is settled; e.g. tyrosine was discovered in 1846 and its constitution only definitely proved in 1882.

This procedure may be adopted for the rapid identification of a known substance; thus by ascertaining whether the substance does or does not contain nitrogen it may be placed in either of the following groups:—

Non-nitrogenous.

Hydrocarbons.
Alcohols, phenols.
Esters, ethers.
Aldehydes.
Ketones.
Acids and salts.
Fats and cholesterol.
Carbohydrates.

Nitrogenous.

Cyanogen compounds.
Amides.
Amines.
Amino acids.
Guanidine compounds.
Purines.
Proteins.

---

[1] The most recent compounds are given in the yearly volumes of the Journals of the English and Foreign chemical societies.

A. Non-nitrogenous.

1. Reduction of Fehling's solution will show aldehyde or carbohydrate. Complex carbohydrates must be hydrolyzed with acid and then tested.

Aldehydes give Schiff's reaction, and the corresponding acid on oxidation.

Carbohydrates give osazones and are optically active.

2. An acid reaction indicates an acid; a neutral reaction and residue on burning away the carbon indicates a salt.

The acid may be characterized by the formation of a typical salt with ferric chloride, calcium chloride, etc.

Phenols react acid, but they give a distinctive coloration with ferric chloride.

3. Esters, fats, cholesterol are soluble in ether. Peculiar physical conditions indicates their nature: esters have sweet smell, fats are greasy. For identification they must be converted into their constituent acid and alcohol. Fats give the acrolein test.

Cholesterol is peculiar in crystalline form and has typical reactions.

4. Alcohols, phenols, ketones. Phenols are acid in reaction.

Alcohols and ketones are neutral. Alcohols form esters with benzoyl chloride, etc., which have fragrant smell. They are oxidized to aldehydes and acids which latter may be identified.

Ketones on oxidation give acids with fewer carbon atoms. The iodoform reaction is not conclusive. They can only be rapidly identified by the exclusion of other groups.

B. Nitrogenous.

1. Amides give off ammonia when boiled with alkali yielding the constituent acid which must be identified.

Test for urea by hypobromite, urea nitrate in concentrated solution.

2. Amines and amino acids do not give off ammonia when boiled with alkali.

Like amides they give off nitrogen when treated with nitrous acid.

Amino acids give salts when boiled with carbonates or oxides, especially copper salt.

Special tests for the amino acids, tyrosine, tryptophane, cystine (S).

3. Uric acid gives the murexide reaction and is soluble in caustic soda.

4. Creatine and creatinine: Jaffe's reaction.

5. Blood and bile are indicated by colour.

Test for blood with spectroscope, conversion into hæmochromogen, hæmin crystals, guaiacum test when boiled.

Test for bile salts and pigments by various tests under bile, especially Gmelin's, Pettenkofer's, Hay's tests.

6. Proteins.

Biuret reaction and other colour reactions. Precipitation by alkaloidal reagents.

(*a*) Coagulable protein. Boil.

(*b*) Metaprotein. Neutralize the solution, if not neutral, and boil.

(*c*) Nucleoprotein, mucin, caseinogen. Solution generally alkaline, therefore acidify.

(*d*) Vitellin, myosinogen. Saline solution: pour into water. (Cf. globulin.)

(*e*) Proteoses, gelatin, peptone, remain in solution.

(*f*) Separation of a mixture.

1. Neutralize exactly and filter off the metaprotein. Suspend the precipitate in water and boil.

2. Half saturate the filtrate with ammonium sulphate. Filter off the globulin and primary proteoses.

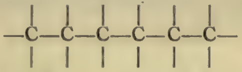
3. Completely saturate the filtrate with ammonium sulphate. Filter off the albumin, secondary proteoses, gelatin.

Dissolve these latter two precipitates in water and boil the solutions; whilst doing so faintly acidify. The globulin and albumin are coagulated. The proteoses and gelatin remain in solution. The gelatin is precipitated by saturation with magnesium sulphate.

HYDROCARBONS.

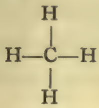
The simplest organic compounds are the hydrocarbons, which consist of carbon united with hydrogen.

Carbon is a tetravalent element, but, unlike other elements, the carbon atom can combine with itself many times thus, 2, 3, 4, 5, 6, etc., carbon atoms can be combined together

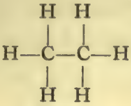


The hydrocarbons are the compounds in which the remaining valencies of the carbon atoms so joined together are satisfied by hydrogen, as for example in

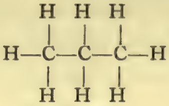
definition of hydro carbon



Methane.



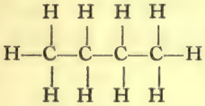
Ethane.



Propane.

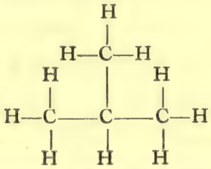
They form a homologous series of compounds in which the member containing one carbon atom more than the preceding one also contains two hydrogen atoms more, i.e. the members differ from one another by CH_2. They possess the general formula C_nH_{2n+2}.

If we continue the process of adding CH_2 to propane, two ways are possible: it may be added to one of the end carbon atoms or to the middle carbon atom. The two compounds



Butane.

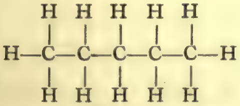
and



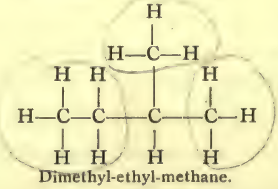
Isobutane or trimethyl-methane.

are thus obtainable. Continuing the process we obtain

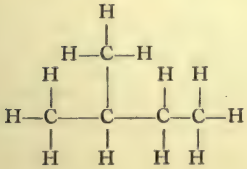
Methane CH_4
Ethane C_2H_6
Propane
Butane
pentane
Hexane



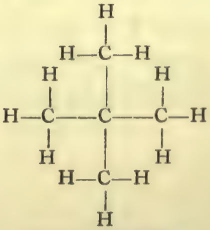
Pentane.



Dimethyl-ethyl-methane.



Dimethyl-ethyl-methane.



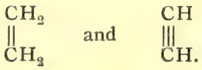
Tetramethyl-methane.

Two or more compounds which have the same empirical composition (C_4H_{10} or C_5H_{12}) but a different structure as represented by the graphic formulæ are therefore possible. Such compounds are known as *isomers.*

The compounds with a straight chain of carbon atoms are termed normal compounds. Those with a branched chain of carbon atoms are regarded as derivatives of methane, the residues CH_3, C_2H_5, C_3H_7, etc., being termed methyl, ethyl, propyl, etc., which shows their origin from the parent hydrocarbon.

In accordance with this theory an enormous number of hydrocarbons are possible; hydrocarbons containing from 1 to 60 atoms of carbon in their molecule are actually known. The theory was advanced and developed to account for their large number.

In addition to these hydrocarbons, there are others which do not possess the general formula C_nH_{2n+2}. The two compounds ethylene C_2H_4, and acetylene C_2H_2, are the typical examples. They are represented by the constitutional formulae,



$$\begin{array}{c} CH_2 \\ \| \\ CH_2 \end{array} \quad \text{and} \quad \begin{array}{c} CH \\ ||| \\ CH. \end{array}$$

The four valencies of the carbon atoms are not completely saturated by hydrogen atoms, and they are therefore termed the unsaturated hydrocarbons.

The hydrocarbons are the basis of the nomenclature and classification of all the carbon compounds. The carbon compounds are classified according as to whether they contain 1, 2, 3, or 4, etc., carbon atoms in their molecule, i.e. whether they are derived from methane, ethane, propane, butane, etc.

The saturated hydrocarbons have thus the suffix *ane;* the unsaturated hydrocarbons are given the suffix *ene* and *ine* respectively. Ethene means 2 carbon atoms and singly unsaturated (a double bond); ethine means 2 carbon atoms and doubly unsaturated (a triple bond). Similarly propene and propine are unsaturated compounds. They contain 3 carbon atoms and isomers are possible.

Numerous important compounds in physiological chemistry contain three carbon atoms, i.e. are derived from propane (or propene). Thus for example acetone, glycerol, lactic acid, and alanine contain three carbon atoms. Uric acid also may be regarded as a derivative of a three carbon atom hydrocarbon, but in this case it is unsaturated.

THE SATURATED HYDROCARBONS.

The majority of the saturated hydrocarbons are natural substances, their chief source being petroleum, which consists of a mixture of them. Two theories have been advanced to account for their formation. According to the first, they are the products of the dry distillation of animal remains; according to the second, they are formed by the action of water upon metallic carbides of which the interior of the earth is supposed to consist. If the former supposition be the correct one, as the most recent work tends to show, they become of still greater interest in biological chemistry.

The lower members of the series of the hydrocarbons (up to 4 carbon atoms) are gases, the next members (containing 5-16 atoms of carbon) are liquids, the remainder (C_{17} and upwards) are solids.

Methane CH_4	Marsh gas or methane is contained in coal gas having been formed by the process of dry distillation. It is formed also by the decomposition of cellulose by bacteria at the bottom of ponds, hence its name. By a similar process of bacterial action it may be produced in the intestines of animals. It is liberated from certain metallic carbides by the action of water.
Ethane C_2H_6 Propane C_3H_8	Are gases and may be prepared by heating sodium propionate and sodium butyrate with soda lime.
Butane Isobutane C_4H_{10}	Are also gases at the ordinary temperature.
Pentanes C_5H_{12} Hexanes C_6H_{14} Heptanes C_7H_{16} Octanes C_8H_{18}	Are liquids, boiling from 38° to 150° C. They are constituents of— Petroleum ether distilling at 50° to 60° C. ,, benzine ,, 70° to 90° C. Ligroin ,, 90° to 120° C.
Nonane C_9H_{20} Decane $C_{10}H_{22}$ Undecane $C_{11}H_{24}$ Dodecane $C_{12}H_{26}$, etc.	Are liquids boiling from 150° to 300° C. They are constituents of— Refined petroleum or kerosene, which is used for illuminating purposes.
Heptadecane $C_{17}H_{36}$, etc.	Are solids melting at 23° to 100° C. Those melting from 30° to 40° C. are known as vaselines; those melting at higher temperatures are paraffin-waxes.

The saturated hydrocarbons are very inert substances; they are not acted upon by concentrated acids or alkalies and on account of their stability they are known as the paraffins from *parum affinis*, little affinity. They are acted upon by the halogens forming substitution products (p. 36).

THE UNSATURATED HYDROCARBONS.

Ethylene is generally prepared by the action of sulphuric acid on ethyl alcohol: ethyl sulphuric acid (p. 43) is first formed and this is converted on heating into ethylene:—

$$C_2H_5HSO_4 = C_2H_4 + H_2SO_4.$$

The higher members of this series are obtained in a similar way to ethylene from the corresponding alcohol by abstraction of water. They are present in small amounts in petroleum from which they are separated by shaking with sulphuric acid. The lower members are gases, the higher liquids and solids.

The unsaturated hydrocarbons in contradistinction to the saturated hydrocarbons are unstable bodies. They combine readily with concentrated sulphuric acid, and with the halogen acids; also with the halogens (see p. 36).

Acetylene is obtained from calcium carbide by the action of water. Thus the various carbides do not give off the same gas when treated with water. (Cf. methane, p. 35.)

Acetylene forms compounds with metals, e.g. cuprous acetylide C_2Cu_2, silver acetylide C_2Ag_2. These compounds are precipitated when acetylene is passed into ammoniacal solutions of cuprous chloride and silver nitrate. In the dry state these compounds are very explosive.

Homologues of acetylene of the general formula C_nH_{2n-2} are known.

Preparation of Methane.

(1) Mix together 1 part of fused sodium acetate and 3 parts of soda lime. Place the mixture in a test tube and heat. Marsh gas is evolved and may be ignited at the mouth of the test tube (dry distillation).

(2) Cover about 2 gm. of aluminium carbide in a test tube with water. Marsh gas is evolved. Collect the gas in an inverted test tube and show that it is inflammable.

Fractional Distillation of Kerosene.

Place about 50 c.c. of refined petroleum in a small dry distilling flask, connect it with a condenser and distil. Notice that the temperature indicated by the thermometer never remains constant for any length of time. This shows that the liquid is a mixture. Collect several fractions boiling within 10 degree ranges of temperature in separate receivers.

By redistilling these fractions in the same way, a pure product may eventually be obtained.

Note.—In practice on a large scale a fractionating column is used. This is simply a device to lengthen the neck of the distilling flask so that the higher boiling fractions are condensed before they reach the condenser and run back into the flask ; only the lower boiling fractions pass over first. Several forms of fractionating column are used ; the simplest consists of a glass tube filled with glass beads (Hempel).

Inflammability of Kerosene.

In about 3 c.c. of refined petroleum in a porcelain basin place a lighted match. It is extinguished. Now warm the liquid on a water bath at 40° C. and again apply a lighted match. The vapours of the petroleum are ignited.

Inertness of the Saturated Hydrocarbons.

Shake up some kerosene and some paraffin wax with :—
(1) Some concentrated sulphuric acid.
(2) Some concentrated nitric acid.
(3) Some caustic soda.
There is no reaction.

CHX

$CHCl_3$

HALOGEN DERIVATIVES OF THE HYDROCARBONS.

The only chemical property of the saturated hydrocarbons is that they are attacked by the halogens yielding halogen substitution derivatives, one atom of hydrogen being progressively replaced by an atom of e.g. chlorine; thus, from methane by the action of chlorine we can obtain

$$CH_3Cl \qquad CH_2Cl_2 \qquad CHCl_3 \qquad CCl_4$$

The trichloro derivative, chloroform, and the corresponding triiodo derivative, iodoform, are both largely used in medicine, the former as an anæsthetic, the latter as an antiseptic. In practice, they are not prepared from marsh gas, but from alcohol or acetone.

Prep of Chloroform

The mechanism of the reaction by which the chloroform and iodoform are formed is probably :—

1. The oxidation of the alcohol to aldehyde (see aldehydes)

$$CH_3 . CH_2OH + O = CH_3 . CHO + H_2O.$$

2. The chlorination of the aldehyde to chloral

$$CH_3 . CHO + 3Cl_2 = CCl_3 . CHO + 3HCl.$$

3. The decomposition of the chloral to chloroform and calcium formate by the calcium hydroxide (p. 50)

$$2CCl_3 . CHO + Ca(OH)_2 = 2CHCl_3 + (HCOO)_2Ca.$$

It has been previously stated that the unsaturated hydrocarbons are unstable substances in comparison with the saturated hydrocarbons. They also differ from the saturated hydrocarbons in their behaviour to the halogens; whereas the saturated hydrocarbons react by substitution, the unsaturated hydrocarbons react by addition thus, e.g. ethylene adds on two atoms of bromine and becomes a saturated compound :—

$$\begin{array}{c} CH_2 \\ \| \\ CH_2 \end{array} + Br_2 = \begin{array}{c} CHBr \\ | \\ CHBr. \end{array}$$

This property is made use of in investigating the nature of fats (p. 53). The unsaturated oleic acid is contained in most if not all fats and its amount is determined by estimating how much iodine is absorbed by the fat.

CHLOROFORM.

(1) Preparation.

Mix together in a one litre flask 100 grammes of fresh bleaching powder, 10 c.c. of alcohol and 20 c.c. of water. Connect it by means of a bent tube to a condenser and receiver. Heat the flask gently on a wire gauze and as soon as the reaction commences remove the flame. The mass in the flask froths considerably and a mixture of chloroform and water distils over, the former forming the lower layer of the distillate. Place the two layers in a separating funnel, shake with caustic soda solution, and draw off the lower layer into a clean flask. Dry the chloroform (which is wet from contact with the water) by allowing it to stand in contact with solid calcium chloride for twelve to twenty-four hours. Then filter it into a clean dry distilling flask and distil off the chloroform. Note its boiling-point of 61° C.

(2) Properties.

(*a*) Shake a small quantity with water in a test tube and add a few drops of silver nitrate. There is no precipitate of silver chloride (unless the chloroform contains free hydrochloric acid).

(*b*) Heat a few drops of chloroform in a test tube with very dilute caustic soda. Sodium chloride and sodium formate are formed. Test for chloride in a small portion of the solution. Neutralize the remainder exactly if it be still alkaline and heat with mercuric chloride solution. A deposit of mercurous chloride and mercury is formed if formate be present.

(*c*) Heat a minute quantity of chloroform with alcoholic potash and a drop of aniline. Note the abominable smell of phenyl isonitrile. (Isonitrile or carbylamine reaction):—

Potash = KOH.

$$CHCl_3 + 3KOH + C_6H_5NH_2 = C_6H_5NC + 3KCl + 3H_2O.$$

Carry out in fume cupboard.

IODOFORM.

Preparation.

Dissolve 4 grammes of potassium carbonate in 25 c.c. water in a small beaker, and add 5 c.c. acetone. Heat to about 70° C. on the water bath. Then slowly add 4 grammes of iodine. As soon as decolorization is complete, filter off the yellow precipitate of iodoform and dry it on a porous plate or between sheets of filter paper.

Prove its identity by determining its melting-point of 119° C.

ALCOHOLS.

Alcohols are hydrocarbons in which a hydrogen atom (or more in the case of the higher members, e.g. glycerol) has been substituted by a hydroxyl or OH group. This relationship is shown:—

1. By the action of water upon the halogen mono-substituted hydrocarbons:—

$$CH_3 . Cl + HOH = HCl + CH_3 . OH.$$

2. By the action of phosphorus pentachloride upon the alcohol:—

$$CH_3 . OH + PCl_5 = CH_3 . Cl + POCl_3 + HCl.$$

All alcohols are designated by the suffix -ol, e.g. methyl alcohol or methanol, ethyl alcohol or ethanol.

Most of the alcohols are natural substances and serve as the starting point for the preparation of other compounds.

METHYL ALCOHOL. $CH_3 . OH$.

Methyl alcohol is the first member of this homologous series. It is a liquid boiling at 66° and is obtained, together with acetone, acetic acid and other substances by the dry distillation of wood. Hence its older name of wood spirit.

ETHYL ALCOHOL, OR ALCOHOL. $CH_3 . CH_2OH$.

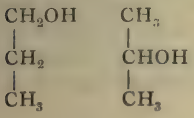
Ethyl alcohol is obtained by the fermentation of sugar by yeast and occurs in all fermented liquids such as wine, beer. It is made chiefly from potatoes, the starch being first converted by acids into the sugar, glucose, which is then fermented and the alcohol distilled off. It is very probable that alcohol is formed in the animal body by a similar process of fermentation.

PROPYL ALCOHOLS. C_3H_8O.

The next member of the series is propyl alcohol, and here we have the first instance of isomerism amongst the alcohols, i.e. two compounds having the same formula but different properties. The two

$CH_3 CH_2 CH_2 OH$

alcohols, propyl alcohol and isopropyl alcohol, are these two compounds. On oxidation the primary propyl alcohol gives propaldehyde, and the secondary, isopropyl alcohol, gives acetone (p. 46). Their formulæ are :—

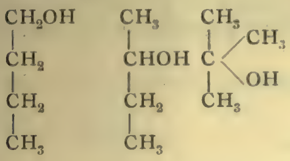


$CH_2OH{-}CH_2{-}CH_3 \qquad CH_3{-}CHOH{-}CH_3$

Note that the secondary alcohol contains the grouping >CHOH, and that the carbon atom to which the OH group is attached is connected to two other carbon atoms.

BUTYL ALCOHOLS. $C_4H_{10}O$.

In the case of the butyl alcohols three isomers are possible, namely :—



$CH_2OH{-}CH_2{-}CH_2{-}CH_3 \qquad CH_3{-}CHOH{-}CH_2{-}CH_3 \qquad (CH_3)_3C{-}OH$

A primary, a secondary and a tertiary, where three carbon atoms are connected to the carbon atom to which the OH group is attached.

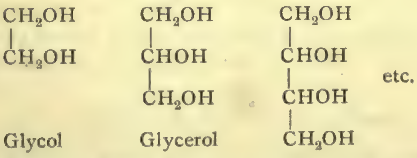
AMYL ALCOHOLS. $C_5H_{12}O$.

Seven isomers are possible, all of which are known, in the case of the alcohols containing five carbon atoms. They are sweet and peculiar smelling liquids boiling at 102°, 112°, 117°, 119°, 128°, 131°, 138° C.

Together with butyl alcohol, two of the amyl alcohols form the principal constituents of fusel oil. They arise from the corresponding amino acid containing one carbon atom more in its molecule.

POLYHYDRIC ALCOHOLS.

Not only can one of the H atoms in a carbon chain be replaced by an OH group but also two, three, four, etc., so long as two OH groups are not attached to only one carbon atom. (See aldehydes, p. 46.) Thus :—



$CH_2OH{-}CH_2OH \qquad CH_2OH{-}CHOH{-}CH_2OH \qquad CH_2OH{-}CHOH{-}CHOH{-}CH_2OH$ etc.

Glycol Glycerol

where primary and secondary alcohols exist side by side in the same substance.

The sugars are derived from complex alcohols of this type.

Glycerol occurs in fats combined with fatty acids in the form of esters.

ETHYL ALCOHOL.

1. Preparation from Wine or Beer.

Place 50 c.c. of wine or 100 c.c. of beer in a distilling flask, filling it not more than two-thirds. Connect the flask to a condenser. Heat the flask steadily with a small flame; if bumping occurs, a piece of unglazed porcelain must be added after cooling. With beer frothing may occur and the distillate which comes over must be redistilled. Collect the distillate in a small flask and show that the portion which comes over first is inflammable.

2. Detection of the Elements.

Set fire to a small quantity of alcohol in a beaker; when burnt cover the beaker with a clock glass and add a few drops of baryta water; shake, and observe the precipitate of barium carbonate—presence of carbon—and that water condenses on the clock glass and cooler portions of the beaker —presence of hydrogen.

Analysis shows that the formula is C_2H_6O and the following equation represents the results of the analysis:—

$$C_2H_6O + 3O_2 = 2CO_2 + 3H_2O.$$

3. Action of Sodium.

To 10 c.c. of absolute alcohol in a small flask add about 0·5 gm. of metallic sodium. Observe that effervescence occurs just as with water, but that the reaction is by no means so violent. Collect the gas evolved in an inverted tube and show that it is hydrogen by burning. When the sodium has dissolved, evaporate the solution to dryness on the water bath. A white solid—sodium ethylate or sodium alcoholate—remains, which is very hygroscopic and is decomposed by water, yielding alcohol which may be recognized by its smell and by the iodoform test:—

$$C_2H_5OH + Na = C_2H_5ONa + H$$
$$C_2H_5ONa + H_2O = C_2H_5OH + NaOH.$$

This reaction shows that one of the hydrogen atoms in alcohol is replaceable by sodium.

4. Action of Phosphorus Pentachloride.

To a small quantity of alcohol add a little phosphorus pentachloride. A vigorous action occurs and hydrochloric acid fumes are evolved. Ethyl chloride and phosphorus oxychloride are the other products. Note the smell of ethyl chloride when the hydrochloric acid fumes cease to be given off. Compare with water. This reaction shows the presence of an hydroxyl or OH group:—

$$C_2H_5OH + PCl_5 = C_2H_5Cl + POCl_3 + HCl.$$

Other reactions show that the C_2H_5 group remains unchanged. It is known as the ethyl radicle, and such groups are known as alkyl groups.

5. Oxidation.

To a little dilute alcohol in a test tube add a few drops of potassium bichromate solution and a little dilute sulphuric acid; warm. Note the characteristic pungent odour of aldehyde and observe that the solution turns green :—

$$C_2H_5OH + O = CH_3CHO + H_2O.$$

6. Iodoform Reaction.

To a drop of alcohol in a test tube full of water, add a solution of iodine in potassium iodide until it is dark brown, and then just enough caustic soda to decolorize the mixture; warm gently. A yellow crystalline precipitate of iodoform, CHI_3, is formed which may be recognized by its characteristic smell (Lieben's reaction).

Note.—This reaction is not absolutely characteristic of alcohol as it may be given by aldehyde, acetone, acetic ester and other substances.

These last two reactions are used as tests for ethyl alcohol.

GLYCEROL.

(*a*) On moistening a piece of paper with a drop of glycerol the paper becomes marked as with a grease spot, but on washing the paper with water and then drying the spot is removed.

(*b*) On heating a few drops of glycerol in a dry test tube with anhydrous phosphoric acid the pungent odour of acrolein is noticed :—

$$CH_2OH . CHOH . CH_2OH = CH_2 : CH . CHO + 2H_2O.$$

Water may also be extracted from glycerol by mixing together 1 part of glycerol and 2 parts of powdered potassium hydrogen sulphate in a test tube and heating the mixture, when the pungent odour of acrolein will be observed.

(*c*) On adding drop by drop an aqueous solution of glycerol (about 20 per cent.) to a 5 per cent. solution of borax to which sufficient phenolphthalein solution has been added to produce a distinct red colour, the red colour is discharged, but on boiling it returns if excess of glycerol has not been used. This is known as Dunstan's test.

Any polyhydric alcohol may give this reaction. Ammonium salts also decolorize the solution, but the colour does not return on heating.

Note that glycerol, like tartaric acid and the sugars, prevents the precipitation of cupric oxide by alkalies.

ESTERS.

Alcohols are like the bases NaOH, KOH in containing an OH group. The bases combine with acids to form salts. Alcohols combine with acids to form esters.

Esters are hydrolyzed by boiling with water, acids, or alkalies. This latter method is known as saponification, i.e. hydrolysis by alkalies. They are thus converted into their constituents, namely alcohol and acid. The recognition of these identifies the ester. In this way the esters which are of frequent occurrence in animals and plants are recognized.

Preparation of Esters.

(*a*) **Ethyl Nitrite**. Warm a little alcohol with copper turnings and a few drops of concentrated nitric acid in a test tube and note the odour of ethyl nitrite (an ester) :—

$$Cu + 3HNO_3 = HNO_2 + Cu(NO_3)_2 + H_2O$$
$$C_2H_5OH + HNO_2 = C_2H_5NO_2 + H_2O$$
$$NaOH + HNO_2 = NaNO_2 + H_2O.$$

Amyl nitrite which is used in medicine is made from sodium nitrite, sulphuric acid, and amyl alcohol.

(*b*) **Ethyl Acetate**. Ethyl acetate is formed when absolute alcohol and glacial acetic acid are heated together. Note the change of odour when 1 c.c. of alcohol and 1 c.c. of glacial acetic acid are boiled in a test tube.

Mix 40 c.c. of concentrated sulphuric acid with the same volume of alcohol in a distilling flask and add slowly 50 gm. of fused sodium acetate. Observe the pleasant fruity odour of ethyl acetate or acetic ester.

Fit a cork carrying a thermometer and connect the distilling flask with a condenser ; distil off the ethyl acetate on the water bath (b. p. 77° C.).

To purify the ester, shake it in a separating funnel with caustic soda solution to remove the acid, draw off the lower layer, and shake the remainder once with water (or better brine) to remove alkali ; again draw off the lower layer and dry the ester by placing it in corked flask for twenty-four hours with solid calcium chloride or potassium carbonate. Then filter into a dry distilling flask and distil off the ethyl acetate :—

$$2CH_3 . COONa + H_2SO_4 = Na_2SO_4 + 2CH_3COOH$$
$$CH_3 . COOH + C_2H_5OH = CH_3 . CO . OC_2H_5 + H_2O.$$

This reaction is used as a test for alcohol, but is not very delicate.

(*c*) **Ethyl Benzoate**. Add to a few drops of alcohol an equal number of drops of benzoyl chloride and then some strong caustic soda. Shake thoroughly for a few minutes and note that the pungent smell of benzoyl chloride has been replaced by the fragrant smell of ethyl benzoate :—

$$C_2H_5 . OH + C_6H_5CO . Cl = C_6H_5CO . OC_2H_5 + HCl.$$

(*d*) **Ethyl Sulphuric Acid.** Pour carefully about 5 c.c. of concentrated sulphuric acid into 15 c.c. of absolute alcohol in a small flask. The mixture becomes hot. Heat the mixture for about fifteen to twenty minutes under a reflux condenser on the water bath (fig. 11).

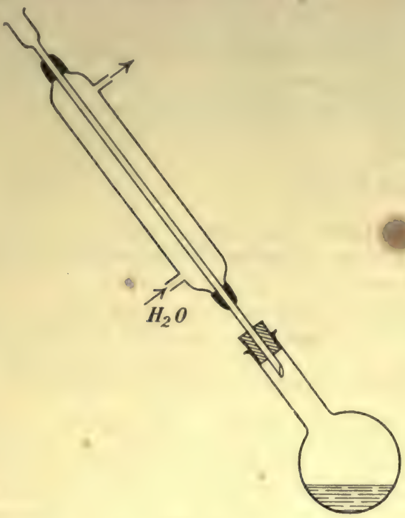


FIG. 11.

Then cool and pour the mixture into 200 c.c. of cold water. Neutralize the solution to litmus by stirring up with barium carbonate. Carbon dioxide is evolved and barium sulphate (insoluble) and barium ethyl sulphate are formed :—

$$C_2H_5 . OH + H_2SO_4 = C_2H_5 . O . SO_2 . OH + H_2O.$$

$$2C_2H_5 . O . SO_2 . OH + BaCO_3 = \left.\begin{array}{l} C_2H_5 . O . SO_2 . O \\ C_2H_5 . O . SO_2 . O \end{array}\right\rangle Ba + CO_2 + H_2O.$$

Heat the neutral solution on the water-bath for ten to fifteen minutes. Filter off the barium sulphate.

(1) Test a portion of the solution with dilute sulphuric acid : a white precipitate of barium sulphate is formed which shows the presence of barium.

(2) Heat another portion of the filtrate with dilute hydrochloric acid for 3 to 5 minutes. Barium sulphate is again precipitated ; the ethyl sulphuric acid is hydrolyzed into alcohol and sulphuric acid :—

$$C_2H_5 . O . SO_2OH + H_2O = C_2H_5OH + H_2SO_4.$$

(3) Convert the remainder of the barium ethyl sulphate into potassium ethyl sulphate by adding a saturated solution of potassium carbonate or a solution of potassium oxalate until no further precipitate is formed :—

$$Ba\left\langle\begin{array}{l} O . SO_2 . OC_2H_5 \\ O . SO_2 . OC_2H_5 \end{array}\right. + K_2CO_3 = BaCO_3 + 2C_2H_5 . O . SO_2 . OK.$$

Filter off the barium carbonate or oxalate and evaporate the filtrate down until a drop removed on a glass rod crystallizes. Allow it to cool. Filter off the crystals and recrystallize from alcohol.

Ethyl chloride (p. 40) may be obtained by treating ethyl alcohol with dry gaseous hydrochloric acid.

Presence of Ethereal Sulphates in Urine.

Ethereal sulphates such as ethyl sulphuric acid occur in urine. Thus :—

Acidify about 20 c.c. of urine with hydrochloric acid and add barium chloride. Filter off the precipitate of barium sulphate. Boil the acid filtrate. A cloudiness is formed due to the decomposition of the ethereal sulphates by the acid into inorganic sulphuric acid, which is precipitated as barium sulphate.

Hydrolysis of Esters.

(*a*) **Ethyl Acetate.**—In a small flask place 10 c.c. of ethyl acetate and add about 80 c.c. of caustic soda solution. Fit a reflux condenser (fig. 11) and boil over a wire gauze for about 15 to 20 minutes. A piece of unglazed porcelain is with advantage added to prevent bumping of the liquid during the heating. The smell of the ester after this time should have disappeared; if not, continue the heating for another 10 to 15 minutes. Alcohol and sodium acetate are formed :—

$$CH_3 . COOC_2H_5 + NaOH = CH_3 . COONa + C_2H_5OH.$$

Prove the presence of alcohol by distilling off at least a quarter of the liquid and applying the iodoform test to a portion of the distillate. Further proof is given by shaking the distillate with anhydrous potassium carbonate (about 10 gm.) which combines with the water and salts out the alcohol, separating the alcohol with a pipette and determining the boiling-point.

The presence of acetic acid is proved by acidifying the contents of the flask with sulphuric acid and again distilling. The distillate will contain acetic acid which is recognized by its odour and acidity and by conversion into basic ferric acetate (see p. 52).

(*b*) **Ethyl Benzoate.** Esters are hydrolyzed more readily by treating with alcoholic caustic soda.

To about 10 c.c. of ethyl benzoate add 50 c.c. of alcoholic caustic soda; warm gently on the water bath and shake the mixture thoroughly. Sodium benzoate is precipitated as it is insoluble in alcohol :—

$$C_6H_5 . COOC_2H_5 + NaOC_2H_5 + H_2O = C_6H_5 . COONa + 2C_2H_5OH.$$

Filter off the sodium benzoate and dissolve it in a small quantity of water. To this solution add concentrated hydrochloric acid. Benzoic acid is precipitated :—

$$C_6H_5 . COONa + HCl = NaCl + C_6H_5 . COOH.$$

Filter off the benzoic acid and wash it till free from mineral acid. Dry it between sheets of paper. Identify it by determining the melting-point of 121° C.

ETHERS.

Ethers are obtained by abstracting water from two molecules of alcohol and may be regarded as the anhydrides of alcohols.

$2 C_2H_5 OH -$ H_2O

$= C_2H_5 OC$

Ether is generally prepared by what is known as the continuous process. Alcohol is allowed to drop slowly upon ethyl sulphuric acid. This is decomposed into ether and at the same time a fresh quantity of ethyl sulphuric acid is formed :—

$$C_2H_5OH + H_2SO_4 = C_2H_5 . O . SO_2 . OH + H_2O.$$
$$C_2H_5 . O . SO_2 . OH + C_2H_5OH = C_2H_5 . O . C_2H_5 + H_2SO_4.$$

MERCAPTANS AND SULPHIDES.

The sulphur compounds corresponding to alcohols are known as mercaptans.

Methyl mercaptan $CH_3 . SH$ is a product of the putrefaction of proteins. It occurs in urine after a diet of asparagus ; hence the peculiar odour of the urine.

The sulphur compound, ethyl sulphide $C_2H_5 . S . C_2H_5$, is another product of the putrefaction of proteins.

Preparation of Ether.

Heat 3 drops of absolute alcohol with two drops of concentrated sulphuric acid. On cooling, ethyl ether can be smelt.

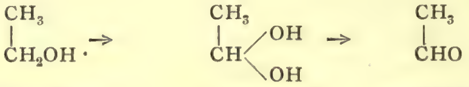
Preparation of Ethyl Mercaptan.

To about 2 c.c. of saturated sodium ethyl sulphate solution, made alkaline with caustic soda, add an equal volume of sodium hydrosulphide solution (33 per cent.) and warm. Ethyl mercaptan is formed, which is recognized by its garlic-like odour :—

$$C_2H_5 . O . SO_2 . ONa + NaSH = C_2H_5 . SH + Na_2SO_4.$$

ALDEHYDES AND KETONES.

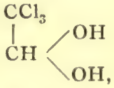
Aldehydes are the first products of the oxidation of primary alcohols, e.g. :—



$$CH_3{-}CH_2OH \rightarrow CH_3{-}CH{<}^{OH}_{OH} \rightarrow CH_3{-}CHO$$

Ethyl Alcohol. Hypothetical. Acetaldehyde.

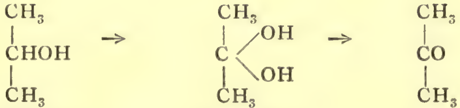
The hypothetical intermediate compound does not exist; it at once loses a molecule of water and is converted into acetaldehyde. With few exceptions, such as chloral hydrate



$$CCl_3{-}CH{<}^{OH}_{OH},$$

two OH groups cannot exist attached to one carbon atom; aldehyde is formed by loss of water. In the same way formaldehyde is formed from methyl alcohol, propaldehyde from primary propyl alcohol, etc.

Ketones are the first products of the oxidation of secondary alcohols, e.g. :—



$$CH_3{-}CHOH{-}CH_3 \rightarrow CH_3{-}C{<}^{OH}_{OH}{-}CH_3 \rightarrow CH_3{-}CO{-}CH_3$$

Isopropyl Alcohol. Hypothetical. Acetone.

The same statements apply here as in the case of the formation of aldehydes.

We thus see that primary alcohols give aldehydes, and secondary alcohols give ketones. Tertiary alcohols on oxidation break down and give no such intermediate product.

The group —CHO is characteristic of aldehydes, that of $>CO$ of ketones.

Aldehydes and ketones are converted by reduction into the corresponding alcohol.

Aldehydes are easily further oxidized into the corresponding fatty acid containing the same number of carbon atoms. They consequently behave as reducing agents—silver salts are reduced to silver, cupric salts to cuprous salts.

Aldehydes are decomposed by alkalies, the lower members yielding resins.

Aldehydes readily undergo condensation. Acetaldehyde is converted into paraldehyde $(CH_3 . CHO)_3$ by the action of concentrated sulphuric acid.

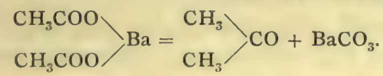
If aldehyde be treated with aqueous potassium carbonate, another condensation product—aldol—is formed :—

$$CH_3 . CHO + CH_3 . CHO = CH_3 . CHOH . CH_2 . CHO.$$

This reaction of aldehyde is analogous to those occurring in plants where, under the action of light and chlorophyll, carbon dioxide is converted into formaldehyde which undergoes aldol condensation into sugar.

Ketones, unlike aldehydes, do not behave as reducing substances. On further oxidation, they are decomposed yielding acids with fewer carbon atoms than the original ketone.

Acetone, the simplest ketone, is obtained in the dry distillation of wood, mixed with methyl alcohol, acetic acid and other products. It is also manufactured by the distillation of calcium or barium acetate :—

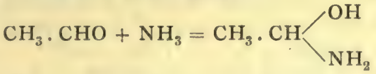


$$\begin{matrix}CH_3COO\\CH_3COO\end{matrix}\!\Big>Ba = \begin{matrix}CH_3\\CH_3\end{matrix}\!\Big>CO + BaCO_3.$$

Aldehydes form additive compounds with :—

1. **Ammonia.**

By passing dry ammonia gas into an ethereal solution of acetaldehyde acetaldehyde-ammonia is formed :—

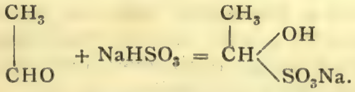


$$CH_3 . CHO + NH_3 = CH_3 . CH\!\Big<\begin{matrix}OH\\NH_2\end{matrix}$$

These compounds are soluble and easily decomposed by acids into aldehyde and ammonia. Formaldehyde behaves differently to other aldehydes in forming hexamethylene tetramine $(CH_2)_6N_4$ (urotropine).

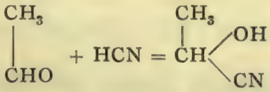
Aldehyde ammonias may be formed in the synthesis of amino acids, etc., in plants (see p. 162).

2. **Sodium Bisulphite.** A crystalline compound is formed when aldehyde is shaken with sodium bisulphite :—



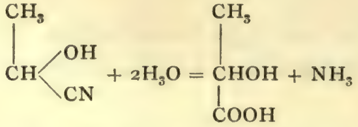
$$\begin{matrix}CH_3\\|\\CHO\end{matrix} + NaHSO_3 = \begin{matrix}CH_3\\|\\CH\end{matrix}\!\Big<\begin{matrix}OH\\SO_3Na.\end{matrix}$$

3. **Hydrogen Cyanide.** Hydrogen cyanide combines with aldehydes forming a cyanohydrin :—



$$\begin{matrix}CH_3\\|\\CHO\end{matrix} + HCN = \begin{matrix}CH_3\\|\\CH\end{matrix}\!\Big<\begin{matrix}OH\\CN\end{matrix}$$

In this way another carbon atom can be added to organic compounds. These cyanohydrins, which contain the CN group, are hydrolyzed by acids or alkalies and converted into the corresponding acid :— (See cyanogen compounds, p. 91)

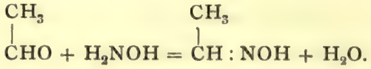


$$CH_3.CH(OH)(CN) + 2H_2O = CH_3.CHOH.COOH + NH_3$$

4. **Phenylhydrazine.** A hydrazone is obtained when aldehyde is treated with phenylhydrazine :—

$$CH_3.CHO + C_6H_5.NH.NH_2 = C_6H_5.NH.N:CH.CH_3 + H_2O.$$

5. **Hydroxylamine.** Oximes are obtained when aldehydes are treated with hydroxylamine :—



$$CH_3.CHO + H_2NOH = CH_3.CH:NOH + H_2O.$$

Ketones form similar additive compounds with sodium bisulphite, phenylhydrazine, and hydroxylamine.

Formaldehyde. H . CHO.

Heat a few drops of methyl alcohol with potassium bichromate and sulphuric acid, and note the pungent, suffocating odour of formaldehyde. The vapours are acid due to formic acid.

This oxidation can be demonstrated as follows: A roll of gauze of copper oxide is heated to redness with a blowpipe flame and dropped into a few c.c. of methyl alcohol in a test tube. The copper oxide is reduced to metallic copper (note colour change), and the vapours of formaldehyde are especially noticeable :—

$$CH_3OH + O = H.CHO + H_2O$$
$$H.CHO + O = H.COOH$$

Acetaldehyde. CH_3 . CHO.

Preparation. Place in a distilling flask of about 200 c.c. capacity, 15 gm. of potassium bichromate and 60 c.c. of water and connect it with a condenser and its receiver and let a strong current of cold water pass through the condenser. Now add a mixture of 20 c.c. concentrated sulphuric acid and 20 c.c. alcohol (90 per cent.), through a separating funnel fastened in the neck of the flask by a cork. The oxidation of the alcohol may commence as soon as the alcohol and acid mixture has been added, but if not, heat the mixture gently with a small flame. A mixture of water, alcohol and acetaldehyde distil over into the receiver. Perform the following reactions with the distillate :—

Reactions.

1. Reduction of metallic oxides in alkaline solution.

use ammoniacal silver nitrate

(*a*) To a little silver nitrate solution add dilute ammonia until the precipitate first formed just re-dissolves. Then add a few drops of dilute aldehyde solution, place in the cold water bath and heat to boiling-point. A mirror of metallic silver forms on the glass.

(*b*) To a little Fehling's solution add a few drops of dilute aldehyde solution and warm. A red or yellow precipitate of cuprous oxide is formed.

Fehling's solution consists of copper sulphate, caustic soda and Rochelle salt (sodium potassium tartrate). When caustic soda is added to copper sulphate a blue precipitate of cupric hydrate $Cu(OH)_2$ is formed, which turns black on boiling. The presence of the Rochelle salt keeps the $Cu(OH)_2$ in solution, and forms a deep blue solution. This solution does not keep, so that it must be freshly made for each experiment. For this purpose two solutions are therefore kept. The one contains the copper sulphate, the other the Rochelle salt and caustic soda. When required for use, equal parts of each are mixed together, and this forms the reagent.

2. To a little aldehyde solution add caustic soda and warm. The liquid becomes darker and a brown resinous substance, aldehyde resin, separates out, with the evolution of a peculiar smell. Aldehyde resin is insoluble in water, but soluble in alcohol and ether.

3. Oxidation to acetic acid. To a little potassium bichromate solution and dilute sulphuric acid add a little aldehyde solution and warm. The solution becomes green and the odour of aldehyde gives place to that of acetic acid.

4. Schiff's test. Bubble a little sulphur dioxide through a solution of magenta or fuchsine until the colour disappears. Then add a few drops of aldehyde solution and observe that the violet colour reappears.

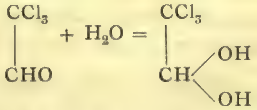
5. To about 1 c.c. of aldehyde add a drop of concentrated sulphuric acid. The aldehyde undergoes polymerization into paraldehyde $(CH_3 . CHO)_3$ which separates out as an oil on adding water.

6. To about 5 drops of aldehyde add about 1 c.c. of cold saturated sodium bisulphite solution and shake vigorously. On standing the additive compound $CH_3 . CH(OH) . SO_3Na$ crystallizes out.

7. To a little phenylhydrazine hydrochloride and sodium acetate (sufficient of each to cover the tip of a knife) dissolved in 10 c.c. of water add a little aldehyde. An oily solution is obtained due to the formation of the phenylhydrazone of acetaldehyde.

Chloral Hydrate and Chloral.

Chloral is formed when alcohol is treated with chlorine (p. 36). It is a liquid with a penetrating smell boiling at 98° C., and polymerizes on keeping like acetaldehyde. It combines with water with evolution of heat forming chloral hydrate :—



$$\begin{array}{c}CCl_3\\ |\\ CHO\end{array} + H_2O = \begin{array}{l}CCl_3\\ |\\ CH\begin{cases}OH\\ OH\end{cases}\end{array}$$

Chloral hydrate is a solid with peculiar smell and melts at 50-51° C. It volatilizes on evaporating its aqueous solution.

1. To some ammoniacal silver nitrate solution add a few drops of chloral solution and warm. Metallic silver is deposited.

2. Place about 2 gm. of chloral hydrate in a test tube and cover it with concentrated sulphuric acid; warm gently. Chloral is formed which floats on the surface. Remove it with a fine pipette, place it in a clean test tube and add a drop of water. Chloral hydrate is reformed with evolution of heat.

3. To some chloral hydrate solution add a little caustic soda and warm gently (with hand). The smell of chloroform is noticed at once :—

$$CCl_3CH(OH)_2 + NaOH = CHCl_3 + HCOONa + H_2O.$$

Reactions of Acetone.

1. (*a*) Acetone does not reduce Fehling's solution.
(*b*) Acetone reduces ammoniacal silver solution only when the mixture is boiled.

2. Acetone treated with caustic soda does not give a resin.

3. Acetone does not polymerize when treated with sulphuric acid.

4. Acetone does not give Schiff's test.

These four reactions are characteristic only for aldehydes. Observe therefore that acetone does not give these reactions.

5. Acetone forms an additive compound with sodium bisulphite in the same way as aldehyde. On adding saturated sodium bisulphite solution to a little acetone the mixture becomes hot, but on cooling the additive compound crystallizes out and in such quantity that the contents of the test tube become quite solid :—

$$CH_3 . CO . CH_3 + NaHSO_3 = (CH_3)_2 : C(OH) . SO_3Na.$$

6. Acetone gives an oily hydrazone with phenylhydrazine hydrochloride and sodium acetate :—

$$CH_3 . CO . CH_3 + C_6H_5 . NH.NH_2 = C_6H_5 . NH.N : C(CH_3)_2 + H_2O.$$

7. On oxidation acetone is converted into acetic and formic acids : i.e. a ketone gives on oxidation an acid containing fewer carbon atoms than itself.

Add a few drops of acetone to about 3 c.c. of water, then add about 1 c.c. of concentrated sulphuric acid and a crystal or two of potassium bichromate and heat. The colour becomes green and the vapours contain acetic and formic acids :—

$$CH_3 . CO . CH_3 + 3O = CH_3 . COOH + H . COOH.$$

8. Acetone gives the iodoform reaction (Lieben's test). To a little dilute acetone solution add caustic soda and iodine in potassium iodide solution. The liquid becomes cloudy, due to the separation of iodoform :—

$$CH_3 . CO . CH_3 + 3I_2 + 4NaOH = CHI_3 + CH_3COONa + 3NaI + 3H_2O.$$

If ammonia be used instead of caustic soda, iodoform is also formed but at the same time nitrogen iodide (black precipitate) is formed. This disappears at once or after standing a short time. Gunning's test.

9. Dilute acetone solution gives with sodium nitroprusside (freshly prepared solution) and caustic soda a blood-red colour. This disappears on standing. On adding excess of acetic acid the colour changes to cherry red. Legal's test.

These last two reactions are of importance for detecting acetone in the urine of diabetic patients.

THE FATTY ACIDS.

The fatty acids are the second oxidation products of the primary alcohols, the aldehydes being the intermediate products. Secondary alcohols and ketones also give rise to them on oxidation, but the number of carbon atoms in their molecule is less than in the original secondary alcohol. Conversely, on reduction fatty acids give aldehydes and primary alcohols, thus:—

$$CH_3 . CH_2OH \leftrightarrows CH_3 . CHO \leftrightarrows CH_3 . COOH.$$

The fatty acids occur widely distributed in nature and combined with glycerol form the fats.

Formic acid can be obtained by oxidizing methyl alcohol with potassium permanganate in the same way as acetic acid from ethyl alcohol (below). It was first prepared by distilling crushed ants with water—hence its name. Its chief use is apparently as a disinfecting agent. On the large scale it is manufactured by heating glycerol with oxalic acid.

Formic acid, like the other fatty acids, is a strong acid and forms salts with metallic oxides and carbonates.

Acetic acid is one of the products made commercially by physiological methods, i.e. by the oxidation of dilute alcohol by means of the micro-organism *mycoderma aceti*, or "mother of vinegar". This is one of our sources of vinegar.

The next members of the series of the fatty acids are propionic, butyric, valerianic, caproic acids with 3, 4, 5, 6 atoms of carbon in their molecule respectively.

These lower members of the fatty acids are volatile with steam, like acetic acid. Hence they are termed the volatile fatty acids. They are separated in this way from the higher members which are not volatile with steam. The principal higher members are palmitic, and stearic acids with 16 and 18 atoms of carbon respectively. Oleic acid has also 16 atoms of carbon in its molecule, but it belongs to the series of unsaturated fatty acids.

The fatty acids are characterized by the presence of the carboxyl or COOH group.

Formic Acid. H . COOH.

1. Boil about 10 c.c. of formic acid with excess of barium carbonate until the solution is neutral. Filter off the excess of barium carbonate and evaporate the filtrate until it commences to crystallize. Allow it to stand for twelve to twenty-four hours. Filter off the crystals of barium formate and dry them on a porous plate or between sheets of filter paper.

2. In the same way prepare lead formate by boiling with litharge.

Reactions of Formates.

Carefully neutralize the formic acid solution with soda or ammonia, or use the solution of barium formate prepared above.

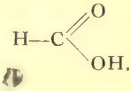
1. Add a little silver nitrate solution and warm. A black deposit of metallic silver is formed.

2. On adding a little mercuric chloride solution and heating a precipitate of mercurous chloride is produced, which, on further heating, may be reduced to metallic mercury.

3. If a few drops of ferric chloride solution be added to the solution of a *neutral* formate a red coloration is produced : on heating a precipitate of basic ferric formate is formed (compare acetates).

4. Add some concentrated sulphuric acid to some *solid* barium or lead formate and heat. Carbon monoxide is evolved which may be ignited at the mouth of the test tube.

The reducing properties as exemplified in (1) and (2) are due to the presence of the aldehyde group HCO in the molecule of formic acid :—



Acetic Acid. CH_3. COOH.

Preparation from Alcohol. Dissolve 14 gm. of potassium permanganate in 200 c.c. of water in a litre flask, then add 8 c.c. of concentrated sulphuric acid. Fit a reflux condenser and add slowly through the condenser a mixture of 5 c.c. of alcohol and 50 c.c. of water. Keep the action moderate. When finished, boil for about 15 minutes. Then distil about three-fourths over. Test the distillate for acetic acid (*a*) by acidity to taste and litmus, (*b*) by exactly neutralizing with soda and adding ferric chloride; the red colour of ferric acetate appears, and on boiling, a brownish-red precipitate of basic ferric acetate is produced.

2. Copper, lead, and other salts are obtained by boiling acetic acid with the corresponding oxides or carbonates until neutral, filtering, and evaporating just as described under formates. Prepare the copper salt.

Chemical Properties of a Higher Fatty Acid.

(*a*) Heat a little "stearine" (commercial "stearine" is a mixture of palmitic and stearic acids) in a test tube; observe that it melts, then boils and gives off white vapours which condense on the side of the tube. There is no smell of acrolein.

(*b*) It is insoluble in water, but soluble in alcohol and ether. Add some of the alcoholic solution to some alcohol to which a drop of caustic soda and a drop of phenolphthalein have been added. The red colour is discharged.

(*c*) On suspending in water and adding caustic soda, drop by drop, it dissolves slowly, forming a clear solution, which froths considerably. On acidifying, the fatty acid is precipitated and can be extracted with ether.

(*d*) Dissolve a little oleic acid in chloroform; to this solution add, drop by drop, a solution of bromine or iodine in chloroform. The colour of the halogen is discharged until the unsaturated acid becomes saturated by absorption of the halogen.

Soaps.

These are the sodium and potassium salts of the higher fatty acids; the former constitute hard soaps, the latter soft soaps.

Dissolve a little soap in water and add excess of dilute sulphuric acid. Filter off the fatty acids, which are liberated and are insoluble in water. Dissolve them in hot caustic soda solution, avoiding excess. Sodium soap is formed. Add to separate portions of the solution a little calcium chloride and magnesium sulphate. Note the curdy precipitates of the calcium and magnesium salts just as are obtained with hard water.

To another portion of the solution add finely powdered sodium chloride; the sodium soap is precipitated as a curdy mass which clings to the sides of the tube. (See Colloids, p. 156.)

THE FATS.

The fats are esters of the higher fatty acids, palmitic, stearic, oleic with glycerol. They occur in most animal and vegetable tissues, together with lecithin. Lecithin has the composition and properties of fats, but contains in addition phosphoric acid and choline. Cholesterol is also usually present.

All these glyceryl esters have similar names wherein the name of the fatty acid describes the nature of the fat, e.g. butyrin, caproin, palmitin, stearin, olein.

Fats are hydrolysed into their constituent fatty acids and glycerol by boiling with water, treatment with steam, and by boiling with acids and alkalies. This latter process of decomposing fats and esters is known as saponification and is a special case of hydrolysis; it was first used in the manufacture of soap, hence the term.

Fats undergo the process of hydrolysis during digestion. They are decomposed by the enzyme lipase in the pancreatic juice and hydrolyzed into their constituents, fatty acids and glycerol. Emulsification (p. 158) occurs in the intestine, where the reaction is alkaline, during the process of the hydrolysis.

Extraction and Estimation of Fat.

As found in nature the fats are contained in animal and vegetable tissues mixed with protein and carbohydrate. Three methods are in use for their separation. The oldest and simplest method consists in melting out the fat from the tissue by simply placing it in a suitable receptacle of muslin or cloth in a warm room when the fat melts and runs out leaving the remainder of the tissue behind. The most modern method consists in pressing out the fat in a hydraulic press; this method is most frequently employed for obtaining the oils from vegetable seeds. The third method consists in extracting the fat by means of suitable solvents, such as benzine, carbon disulphide. Alcohol and ether are not usually employed for this purpose, but they are generally used when an estimation of fat in a tissue is required.

In the estimation of fat, the tissue must first be dried: this is effected by mixing a known weight of the material with clean dry sand or other suitable absorbing medium and then heating it for 1-2 hours in a steam oven. In the case of milk, it is most convenient to absorb a known weight (or volume) in clean fat-free filter paper, which is made into a small roll, and to dry this. The dried material is then placed in a paper thimble of suitable size and this is extracted with ether for 2-3 hours in a Soxhlet apparatus which allows of a continual extraction for that time without constant attention. The Soxhlet apparatus (fig. 12) consists of (1) a small dry flask, the weight of which has been accurately determined, (2) a special extracting tube into which the thimble and material is placed, (3) a short condenser. The extracting tube is composed of a wide piece of glass tubing like a test tube fused at its closed end to a narrower piece of glass tubing which is cut off at an angle at its other extremity. Just below the join of these pieces of tubing a glass side tube is fused into the narrower piece; its other end is fused into the wider piece at the upper end. At the base of the wide tube, on the other side of the apparatus, one end of a narrow syphon tube is attached; its other end is fused to the narrow piece, through which it passes and opens just above the angular extremity. The narrow end of this tube is fastened into the flask; the condenser is attached to the wider end. Ether is placed in the flask which is gently heated. The volatilized ether passes through the side tube and reaches the condenser. The condensed drops fall upon the thimble and cover it. When completely covered the ether is syphoned off and returns to the flask and the process is repeated. It remains to distil off the ether from the flask, dry at 100° C. and weigh. The difference in weight gives the amount of fat in the known weight of tissue.

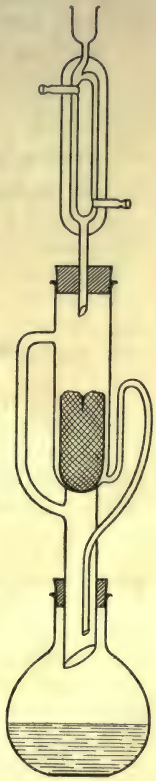


FIG. 12.

Solubility.

Fats resemble the fatty acids in their solubilities. Fats are not soluble in dilute cold caustic soda. Perform the following experiments with lard and olive oil :—

(*a*) Treat with water. They do not dissolve. The lard melts on warming.

(*b*) Treat with alcohol. Some of the olive oil dissolves, so also does the lard, but this may require heating. Pour a few drops of the solution on filter paper and note the grease stain.

(*c*) Treat with ether. Both lard and olive oil are soluble in ether. Put a few drops of the ethereal solution on filter paper. A greasy stain is left.

Hydrolysis.

(*a*) **Butter.** Heat a little butter with a small quantity of alcoholic soda until a clear yellow solution is obtained. Add a little water (no oil drops should be seen) and heat to expel the alcohol. Then acidify with dilute sulphuric acid and warm again, when the smell of butyric acid (and caproic acid) will be noticed. The butyric acid may be obtained by distilling as described under the preparation of acetic acid. The higher fatty acids are also contained in butter, but they do not distil with the steam.

(*b*) **Olive Oil.** Dissolve a little olive oil in twice its quantity of ether, then add five times its volume of alcoholic soda solution (1 gm. sodium in 50 c.c. alcohol). Cork and let the mixture stand. The mixture solidifies, forming a jelly. Complete saponification has occurred, and soap has been formed. Dissolve it in water and test with calcium chloride as directed below.

(*c*) **Lard.** Boil a small quantity of lard with 10 to 20 c.c. of alcoholic soda for about five minutes. The fat is converted into soap (= sodium stearate, etc.). Then pour the solution into an evaporating basin and evaporate off the alcohol. Add about 10 to 20 c.c. of water; no oil drops should be seen; if present, saponification (= hydrolysis by alkali) is incomplete and must be completed by boiling with more alcoholic soda. Acidify with dilute sulphuric acid. A precipitate of fatty acid is formed from the soap. Filter it off through a wet paper and wash it free from sulphuric acid. Keep the filtrate A to test for glycerol. Prove that the precipitate consists of fatty acid :—

1. Dissolve a little in ether and add it to alcohol containing a drop of phenolphthalein and a drop of dilute soda. The red colour disappears.

2. Dissolve some in caustic soda. A soap lather is produced on shaking some up with warm water. Add some sodium chloride to another portion, the soap is precipitated and rises to the surface (*salted out*). To a third portion add some calcium chloride. A precipitate of calcium soap (calcium stearate, etc.) is produced.

small quant. heat slowly

3. Heat some with acid potassium sulphate. No smell of acrolein (from glycerol) is produced if it has been washed free from glycerol. Compare with the smell given by the fat when treated in the same way.

Detection and Isolation of Glycerol.

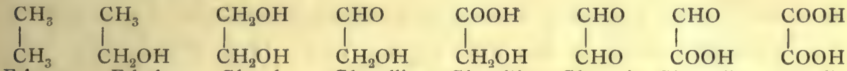
Neutralize the filtrate A from the precipitate of fatty acid and evaporate it to a syrup on the water bath. Mix this with alcohol which precipitates inorganic sodium sulphate. Pour off the alcoholic solution of glycerol and again evaporate. Test for glycerol by heating with acid potassium sulphate and noting the smell of acrolein.

HYDROXY AND DIBASIC ACIDS.

So far only simple organic compounds, compounds which are either alcohol, aldehyde, or acid have been studied. The more complex substances are compounds of the nature of alcohol and aldehyde, alcohol and acid, etc., combined. They are derived from hydrocarbons containing two or more carbon atoms in the same way as methyl alcohol and acetic acid are derived from methane and ethane.

Compounds Containing Two Carbon Atoms.

The following series of compounds can be obtained from ethane :—



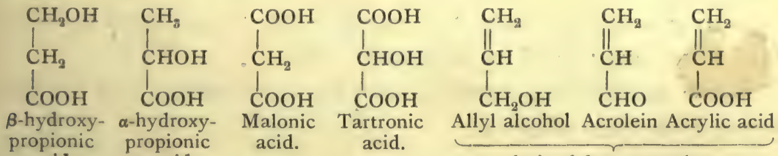
$\begin{array}{c}CH_3\\ \vert\\ CH_3\end{array}$	$\begin{array}{c}CH_3\\ \vert\\ CH_2OH\end{array}$	$\begin{array}{c}CH_2OH\\ \vert\\ CH_2OH\end{array}$	$\begin{array}{c}CHO\\ \vert\\ CH_2OH\end{array}$	$\begin{array}{c}COOH\\ \vert\\ CH_2OH\end{array}$	$\begin{array}{c}CHO\\ \vert\\ CHO\end{array}$	$\begin{array}{c}CHO\\ \vert\\ COOH\end{array}$	$\begin{array}{c}COOH\\ \vert\\ COOH\end{array}$
Ethane.	Ethyl alcohol.	Glycol.	Glycollic aldehyde.	Glycollic acid.	Glyoxal.	Glyoxylic acid.	Oxalic acid.

After ethyl alcohol, oxalic acid, which is obtained by the oxidation of sugar, cellulose, etc., is the most important compound in this series. Glyoxylic acid is one of the reagents which is used in testing for the proteins. Glycollic aldehyde is the first representative of the group of carbohydrates.

Compounds Containing Three Carbon Atoms.

The number of compounds which can be derived from propane are more numerous than those from ethane, since the extra carbon atom introduces further possible combinations and permutations. The principal compounds are :—

CH_3
CH_2
CH_3



$\begin{array}{c}CH_2OH\\ \vert\\ CH_2\\ \vert\\ COOH\end{array}$	$\begin{array}{c}CH_3\\ \vert\\ CHOH\\ \vert\\ COOH\end{array}$	$\begin{array}{c}COOH\\ \vert\\ CH_2\\ \vert\\ COOH\end{array}$	$\begin{array}{c}COOH\\ \vert\\ CHOH\\ \vert\\ COOH\end{array}$	$\begin{array}{c}CH_2\\ \Vert\\ CH\\ \vert\\ CH_2OH\end{array}$	$\begin{array}{c}CH_2\\ \Vert\\ CH\\ \vert\\ CHO\end{array}$	$\begin{array}{c}CH_2\\ \Vert\\ CH\\ \vert\\ COOH\end{array}$
β-hydroxy-propionic acid.	α-hydroxy-propionic acid (lactic acid).	Malonic acid.	Tartronic acid.	Allyl alcohol	Acrolein	Acrylic acid

derived from propylene.

The first two compounds are isomers; in their nomenclature the position of the hydroxyl group is indicated by the Greek letters *α* and *β*, the lettering or numbering being commenced at the carbon atom next to the COOH group in the chain.

ydroxy
ropionic

Lactic Acid. $CH_3 . CHOH . COOH$.

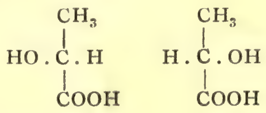
Lactic acid is a syrupy liquid, which is soluble in water, alcohol and ether. It is the product formed by the fermentation of sugar by the lactic acid bacteria; hence its presence in milk when it turns sour. This is its method of preparation, i.e. by biological means. It is contained in muscle and other organs, especially after activity.

Lactic acid is the simplest compound which exhibits the phenomenon of circular polarization. Most natural compounds exhibit this phenomenon. Circular polarization is the property of rotating a ray of polarized light to either the right or the left.

According to its source lactic acid may be either dextrorotatory or laevorotatory, or inactive. Thus lactic acid from muscle—sarcolactic acid—is dextrorotatory. Certain bacteria produce laevorotatory lactic acid. Fermentation lactic acid is inactive.

The examination of natural substances which exhibit circular polarization has shown that they all contain one or more asymmetric carbon atoms, i.e. carbon atoms combined with four different groups. By representing the carbon atom as a regular tetrahedron, and placing the different groups at the four apices, Van't Hoff and Le Bel have given us an explanation of the phenomenon. Adopting any arrangement of the groups round the tetrahedron, a reverse arrangement is represented by its image in a mirror. If therefore one variety is represented by the first arrangement, the mirror image of the figure represents the opposite variety. Projected on to a plane surface the following formulæ are then obtained:—

Explanation of
circular polarization



$$\begin{array}{c} CH_3 \\ | \\ HO . C . H \\ | \\ COOH \end{array} \qquad \begin{array}{c} CH_3 \\ | \\ H . C . OH \\ | \\ COOH \end{array}$$

One figure will represent laevo or *l*-lactic acid, the other figure dextro or *d*-lactic acid. A mixture of the two in equal quantities represents inactive or *dl*-lactic acid. This can be proved by the separation of inactive lactic acid into its constituent *d*- and *l*-forms by the fractional crystallization of its strychnine salt.

These optical isomers are known as stereoisomers and the phenomenon as stereoisomerism.

Compounds Containing Four Carbon Atoms.

The vegetable acids, malic and tartaric, and the two acids β-hydroxybutyric and acetyl-acetic, which are found in the urine in cases of diabetes, are the chief biological representatives of this group of compounds. Malic acid is monohydroxy-succinic acid; tartaric acid is dihydroxy-succinic acid. Succinic acid is the dibasic acid of this series, oxalic acid being the first member:—

(ethane) CH_3 $COOH$.

CH_3
CH_2
CH_2
CH_3

CH_3
CH_2
CH_2
$COOH$

$$\begin{array}{ccccc}
\mathrm{COOH} & \mathrm{COOH} & \mathrm{COOH} & \mathrm{CH_3} & \mathrm{CH_3} \\
| & | & | & | & | \\
\mathrm{CH_2} & \mathrm{CH_2} & \mathrm{CHOH} & \mathrm{CHOH} & \mathrm{CO} \\
| & | & | & | & | \\
\mathrm{CH_2} & \mathrm{CHOH} & \mathrm{CHOH} & \mathrm{CH_2} & \mathrm{CH_2} \\
| & | & | & | & | \\
\mathrm{COOH} & \mathrm{COOH} & \mathrm{COOH} & \mathrm{COOH} & \mathrm{COOH}
\end{array}$$

Succinic acid. Malic acid. Tartaric acid. β-hydroxybutyric acid. Acetylacetic acid.

Malic acid is optically active like lactic acid, i.e. contains one asymmetric carbon atom; so also is β-hydroxybutyric acid.

Tartaric acid is also optically active, but in its molecule two asymmetric carbon atoms are present. The same forms exist here as in the case of lactic acid—a dextro, a laevo, and an inactive; but, on account of the presence of the two asymmetric carbon atoms another inactive form can exist. The two asymmetric carbons have the same groups attached to them; if the arrangements of these groups round each carbon atom are the same, an optically active form will result; but if different an inactive form will result: in the former case both asymmetric carbon atoms are rotating to the right or to the left; in the latter case the one carbon atom rotates in one direction as much as the other carbon atom rotates in the other direction and they neutralize one another. Internal compensation occurs.

This inactive acid is known as mesotartaric acid. It differs from the mixture of equal parts of *d*- and *l*- (known as racemic acid) by the fact that it can never be separated into its two constituents by the special methods which have been employed. One of these methods is biological and was discovered by Pasteur. It consists in the action of moulds which destroy the one form and leave the other unchanged; another method is by the fractional crystallization of the salts with alkaloids, e.g. strychnine as seen in the case of lactic acid.

The following formulæ represent the projections of the tetrahedra arranged to show the stereoisomerism of the tartaric acids:—

$$\begin{array}{ccc}
\mathrm{COOH} & \mathrm{COOH} & \mathrm{COOH} \\
| & | & | \\
\mathrm{HCOH} & \mathrm{HOCH} & \mathrm{HCOH} \\
| & | & | \\
\mathrm{HOCH} & \mathrm{HCOH} & \mathrm{HCOH} \\
| & | & | \\
\mathrm{COOH} & \mathrm{COOH} & \mathrm{COOH} \\
\underbrace{d\text{-tartaric acid} \quad l\text{-tartaric acid}}_{\text{Inactive tartaric acid.}} & & \text{Mesotartaric acid.}
\end{array}$$

Citric Acid.

Citric acid is another hydroxy acid which occurs in nature: it is the acid constituent of lemons and oranges and other fruits; it is present also in milk. Six carbon atoms are contained in its molecule, five being in a normal straight chain. Its formula is:—

$$\begin{array}{l}
\mathrm{CH_2 . COOH} \\
| \\
\mathrm{C(OH) . COOH} \\
| \\
\mathrm{CH_2 . COOH}
\end{array}$$

Preparation and Properties of Glyoxylic Acid.

Glyoxylic acid is prepared most conveniently by the reduction of oxalic acid; sodium amalgam has been most frequently employed, but Benedict suggests the use of magnesium.

omit.

Place about one gm. of powdered magnesium in a small flask and just cover it with distilled water. Then add slowly 25 c.c. of saturated oxalic acid solution. The reaction proceeds rapidly with liberation of heat and the flask should be cooled with water. Filter off the insoluble magnesium oxalate which is formed, and test the solution for aldehyde (Schiff's reaction).

As reagent for proteins, acidify with acetic acid and make up to 100 c.c. with distilled water and preserve in a stoppered bottle.

Reactions of Oxalic Acid.

omit.

1. Heated on platinum, nickel, or a crucible lid, oxalic acid is volatilized without charring (see p. 2).

2. Dissolve a crystal in concentrated sulphuric acid and warm: Carbon dioxide and carbon monoxide are evolved:—

8

$$COOH.COOH = CO_2 + CO + H_2O.$$

3. Acidify a little oxalic acid solution with sulphuric acid and add some potassium permanganate solution. It is decolorized. The oxalic acid is oxidized with liberation of carbon dioxide:—

$$COOH.COOH + O = 2CO_2 + H_2O.$$

4. Calcium oxalate is precipitated when calcium chloride is added to a solution of a neutral oxalate. It is insoluble in acetic acid, but soluble in mineral acids.

Lactic Acid.

s typical hydroxy-acid.

Preparation. To a solution of 50 gm. of cane sugar in 500 c.c. of water 20 gm. of chalk and 20 to 30 c.c. of sour milk (which contains lactic acid bacteria) are added and the mixture is kept at 40° C. for 6 to 8 days with occasional shaking. Calcium lactate is formed. The chalk is added to neutralize the lactic acid which hinders the growth of the bacteria.

omit →

Boil up a portion of this solution (to kill bacteria), filter and evaporate it on the water bath till crystallization commences. Filter off the calcium lactate; press it between sheets of filter paper and recrystallize it from hot water. Prove the presence of lactic acid in this salt.

Reactions.

1. Boil a little concentrated lactic acid solution for some time with excess of zinc carbonate and filter whilst hot. On cooling zinc lactate crystallizes out if the solution be sufficiently concentrated. The zinc salt is the most characteristic salt.

2. To a little dilute ferric chloride solution (scarcely coloured) add a few drops of dilute lactic acid. The colour becomes yellow (ferric lactate).

3. Uffelmann's test for lactic acid. To a little (2 per cent.) phenol solution add ferric chloride till the colour is distinctly violet, and then a little lactic acid solution. The colour changes to yellow, due to the formation of ferric lactate.

4. Shake up with ether a little lactic acid solution acidified with sulphuric acid. When the two liquids have separated, pour off the ether into a clean test tube and shake with dilute ferric chloride solution. As the ether extracts the lactic acid from the solution the colour will become yellow.

5. Lactic acid gives the iodoform reaction.

Reactions of Tartaric Acid.

1. Heat a little tartaric acid in a test tube. It melts and then chars, giving off the odour of burnt sugar.

2. A precipitate of potassium hydrogen tartrate is formed when potassium chloride and acetic acid are added to a (not too dilute) solution of a tartrate. omit.

3. Tartaric acid prevents the precipitation of metallic hydroxides by caustic soda. read.

(*a*) To a little ferric chloride solution add caustic soda and then some sodium potassium tartrate solution. A reddish-brown precipitate of ferric hydroxide is formed, which dissolves on adding the tartrate, and gives a yellow-brown solution.

(*b*) Carry out the same reaction using copper sulphate instead of ferric chloride. A dark blue solution results. This property is used in the preparation of Fehling's solution.

4. Add a little silver nitrate to some sodium potassium tartrate solution. A white precipitate is formed, which is soluble in ammonia and on heating deposits metallic silver.

5. On adding calcium chloride to a *cold* solution of a neutral tartrate (sodium potassium tartrate) a white precipitate of calcium tartrate is formed, which is soluble in acetic acid and caustic soda; on boiling the latter solution, it reappears.

Reactions of Acetyl Acetic Acid (or Aceto-Acetic Acid).

1. Dilute ferric chloride added drop by drop to a solution of acetyl acetic acid gives a claret-red colour (Gerhardt's reaction). This disappears on boiling. Distinction from salicylic acid, antipyrine.

2. Acidulate some of the solution with sulphuric acid and extract it with ether. Separate off the ether and test it for acetyl acetic acid by shaking with ferric chloride. omit.

3. On adding a few drops of a freshly prepared solution of sodium nitroprusside and rendering alkaline with caustic soda, a deep red colour is formed. The colour becomes reddish-purple on acidifying with acetic acid. (Legal's test). Compare with Weyl's test for creatinine (p. 122).

4. On heating with dilute alkali or acid, acetyl acetic acid is decomposed yielding acetone. This may be detected by its odour. It is identified by distilling and testing the first portion of the distillate with the iodoform test.

Reactions of Citric Acid. Compare with those of tartaric acid.

1. Potassium chloride and acetic acid do not give a precipitate with a citrate. omit. read.

2. Its silver salt when dissolved in ammonia does not reduce, giving metallic silver.

3. Calcium chloride when added to a neutral citrate does not give a precipitate until the solution is boiled.

THE CARBOHYDRATES.

Compounds of the nature of alcohols, primary and secondary, and at the same time aldehyde or ketone, form the very important class of compounds termed the carbohydrates or sugars. Their reactions depend upon the presence of these groups combined together in a single molecule. Their empirical formula shows that they consist of carbon and water $C_n(H_2O)_n$. This class of compounds contains members with 2, 3, 4, 5, 6, 7, 8, 9, etc., carbon atoms, the chief physiological representatives being the members with six carbon atoms. It was formerly supposed that only these members belonged to the class of sugars and it may be convenient to term these six carbon atom compounds the sugars whilst the whole group is termed the carbohydrates. The various members of the group contain one or more asymmetric carbon atoms; consequently they are optically active. In the case of the six carbon atom members four asymmetric carbon atoms are present in the molecule and sixteen stereoisomers are possible. Only four of these are found in nature, and are important physiologically, namely, glucose, fructose, galactose, mannose. Most of the other stereoisomers have been prepared in the laboratory by Emil Fischer. The members of the carbohydrate group are distinguished by the suffix -ose. Combinations together of 2, 3, 4 and more of these six carbon atom compounds also occur in nature forming the reserve food material of plants and animals. According as they contain 2, 3, 4, etc., units they are termed disaccharides, trisaccharides, polysaccharides, the single unit compound being termed a monosaccharide. Lactose, cane sugar, and maltose are disaccharides, raffinose is a trisaccharide, starch, glycogen, inulin are polysaccharides, the number of units contained in them being at present unknown. Cellulose, the gums, etc., are polysaccharides containing five carbon atom units. These complex compounds are converted into the simple monosaccharides by hydrolysis with acids.

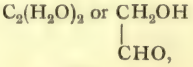
Starch is the reserve material of plants and occurs chiefly in the seeds.

Glycogen is the reserve carbohydrate material of animals and occurs chiefly in the liver; it is also present in muscles and other organs. It is a white powder soluble in cold water giving an opalescent solution.

Cellulose forms the skeletal structure of plants.

Lactose is present in milk, cane sugar or sucrose in the cane and beet. Maltose is a product of hydrolysis of starch. Raffinose is formed under certain conditions in the beet. Glucose and fructose occur in fruits.

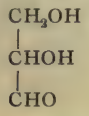
Formaldehyde CH_2O has the empirical formula of a carbohydrate and is in reality the first member of the series. Glycollic aldehyde,



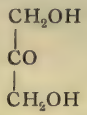
$$C_2(H_2O)_2 \text{ or } \begin{array}{l} CH_2OH \\ | \\ CHO, \end{array}$$

as it contains a primary alcohol group and an aldehyde group is essentially the first member. It is derived, like all the members of the carbohydrate group, by oxidation of the corresponding dihydric alcohol, glycol (pp. 39, 57).

Glyceric aldehyde and dihydroxy acetone $C_3(H_2O)_3$



$$\begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ CHO \end{array}$$



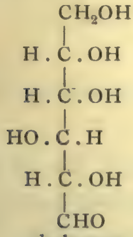
$$\begin{array}{c} CH_2OH \\ | \\ CO \\ | \\ CH_2OH \end{array}$$

are derived from glycerol. They are respectively the first examples in the series of an aldose and a ketose. As they contain three carbon atoms they are termed trioses.

The tetroses contain four atoms of carbon, the pentoses five, the hexoses six, and so on. The number of possible isomers increases as the number of carbon atoms increases.

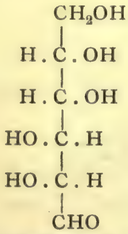
Pentoses have been found in the urine in different conditions; they are also contained in nucleic acid, which forms part of the molecule of the nucleoproteins, and from which they are obtained on hydrolysis. Like other carbohydrates, their chief source is vegetable. The principal representatives are arabinose and xylose which are both aldoses and which differ in the arrangement of the groups around their asymmetric carbon atoms. Arabinose is obtained from gum arabic and xylose from straw or pine wood.

Of the hexoses, glucose, galactose, and mannose are aldoses; fructose is a ketose. The three former differ only in their stereoisomerism. Their formulæ are:—



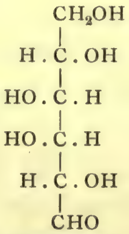
$$\begin{array}{c} CH_2OH \\ | \\ H.C.OH \\ | \\ H.C.OH \\ | \\ HO.C.H \\ | \\ H.C.OH \\ | \\ CHO \end{array}$$

d-glucose.



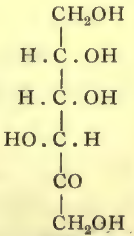
$$\begin{array}{c} CH_2OH \\ | \\ H.C.OH \\ | \\ H.C.OH \\ | \\ HO.C.H \\ | \\ HO.C.H \\ | \\ CHO \end{array}$$

d-mannose.



$$\begin{array}{c} CH_2OH \\ | \\ H.C.OH \\ | \\ HO.C.H \\ | \\ HO.C.H \\ | \\ H.C.OH \\ | \\ CHO \end{array}$$

d-galactose.



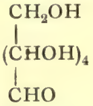
$$\begin{array}{c} CH_2OH \\ | \\ H.C.OH \\ | \\ H.C.OH \\ | \\ HO.C.H \\ | \\ CO \\ | \\ CH_2OH \end{array}$$

d-fructose.

$C_6H_{12}O_6$
72
12
16
180 gm

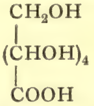
The constitution of these sugars is proved by their reduction to the corresponding hydrocarbon hexane and by oxidation. Fructose, on oxidation, is broken down at the ketone group (compare acetone). Glucose, on oxidation, gives firstly gluconic acid and then saccharic acid. In the animal body another oxidation product of glucose is formed, namely glycuronic acid, the primary alcohol group being converted into the carboxylic acid group, whilst the aldehyde group is unchanged. This compound is also classed among the carbohydrates.

Glycuronic acid occurs in the urine under certain conditions, e.g. after the administration of chloral hydrate, as urochloralic acid. Other substances also pass out into the urine combined with glycuronic acid. Its best source is euxanthic acid, which, combined with magnesium and calcium, constitutes the yellow pigment, Indian yellow. This is obtained from cow's urine after feeding with mango leaves. We have thus



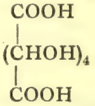
$$\begin{array}{c} CH_2OH \\ | \\ (CHOH)_4 \\ | \\ CHO \end{array}$$

Glucose.



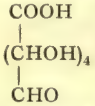
$$\begin{array}{c} CH_2OH \\ | \\ (CHOH)_4 \\ | \\ COOH \end{array}$$

Gluconic acid.



$$\begin{array}{c} COOH \\ | \\ (CHOH)_4 \\ | \\ COOH \end{array}$$

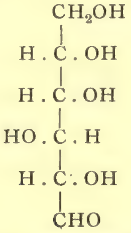
Saccharic acid.



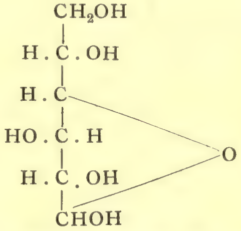
$$\begin{array}{c} COOH \\ | \\ (CHOH)_4 \\ | \\ CHO \end{array}$$

Glycuronic acid.

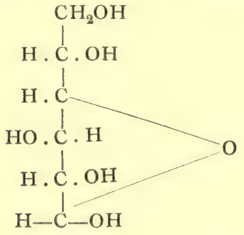
Glucose is represented above as a hydroxy-aldehyde, but this constitution does not entirely explain all its chemical and physical properties. A freshly prepared solution of glucose shows a higher rotatory power than the solution when it has been kept for some hours. Tanret has isolated from glucose solutions under certain conditions two compounds, one of which, α-glucose, has a high initial rotatory power of 110°, the other, β-glucose, a low one of 19°. On being kept in solution both compounds give a solution of the same rotatory power of 52·5°. Two isomeric methyl, etc., derivatives are also obtainable from glucose (see glucosides). Glucose in its chemical behaviour is also less active than is expected. These properties are explained by assuming that glucose has a γ-lactone formula, i.e. a formula in which four carbon atoms and an oxygen atom are included in a ring, thus



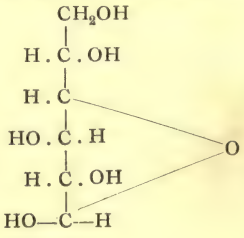
$$\begin{array}{c} CH_2OH \\ | \\ H.C.OH \\ | \\ H.C.OH \\ | \\ HO.C.H \\ | \\ H.C.OH \\ | \\ CHO \end{array}$$



Under these conditions the carbon atom to which the aldehyde group is attached becomes asymmetric. Two stereoisomeric forms are therefore possible. These represent α- and β-glucose :—



α-glucose.

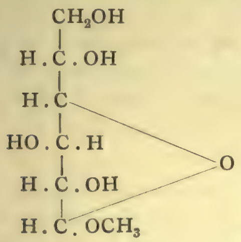


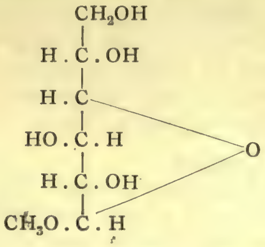
β-glucose.

In solution both forms pass into the aldehyde form.

Glucosides.

In addition to these compounds there also occur in nature a large number of combinations of glucose, or more rarely of other sugars, with various classes of organic compounds, especially those belonging to the aromatic series. These are the glucosides. Their chemical properties resemble those of cane sugar and the polysaccharides. Glucosides have also been prepared in the laboratory from glucose, mannose, maltose, etc.; two isomers are generally thus obtained, termed the *α*- and *β*-glucosides. The most important are the *α*- and *β*-methyl glucosides which are prepared by the action of hydrochloric acid upon a solution of glucose in methyl alcohol. They are derived from *α*- and *β*-glucose by the replacement of the hydrogen atom in the hydroxyl group attached to the carbon atom which possesses aldehydic properties:—





The natural compounds are all derivatives of *β*-glucose, e.g. salicin, arbutin, phloridzin. The most interesting is amygdalin, which is a combination of benzaldehyde, hydrogen cyanide and two molecules of glucose in the form of a disaccharide.

The principal carbohydrates are therefore:—

Monosaccharides.	Trioses. Pentoses. Arabinose, xylose. Hexoses. Glucose, fructose, galactose, mannose. Glycuronic acid.
Disaccharides.	Maltose, lactose, cane sugar.
Polysaccharides.	Starch, dextrin, glycogen, inulin.
Glucosides.	Salicin, phloridzin, amygdalin.

REACTIONS OF THE MONOSACCHARIDES.

The reactions of glucose may be taken as typical of the reactions of carbohydrates.

A. GLUCOSE.

1. Formation of esters (alcohol reaction). Shake up a little glucose solution with benzoyl chloride and excess of caustic soda. A precipitate is formed consisting of the benzoic acid ester of glucose :—

$$C_6H_7O(OH)_5 + 5C_6H_5.COCl = C_6H_7O(C_6H_5.CO.O)_5 + 5HCl.$$

2. Action of alkali (aldehyde reaction). **Moore's test.** Boil a small portion with a little caustic soda. The solution turns yellow, then dark brown and smells of caramel; the small becomes more distinct on acidifying with dilute sulphuric acid.

3. Reduction of metallic oxides in alkaline solution (aldehyde reaction).

(*a*) **Silver.**

Add a little sugar solution to some ammoniacal silver nitrate solution (prepared by adding dilute ammonia to silver nitrate until the precipitate first formed is just redissolved) and warm in the water bath. A mirror of metallic silver gradually forms.

(*b*) **Copper.**

(i) **Trommer's test.** Make some of the solution alkaline with caustic soda, and add copper sulphate, drop by drop, shaking after each addition until the solution becomes deep blue. (Excess of $CuSO_4$ causes the precipitation of $Cu(OH)_2$ i.e. it is no longer dissolved by the sugar solution. A few small crystals of Rochelle salt (NaKTart) cause this to redissolve (see Fehling's test).) Heat nearly to boiling. A yellowish-red precipitate of cuprous oxide is formed.

(ii) **Fehling's test.** To a little of the sugar solution add equal quantities of Fehling's solution (a) $CuSO_4$; (b) NaOH + NaKTart. Note that no precipitate is formed with the excess of copper sulphate (Cf. Trommer's test.) Heat to boiling; cuprous oxide is precipitated.

Note. 1. This solution is used in the estimation of sugars. (Fehling's method.)

2. The presence of ammonia and ammonium salts hinder the reaction as the cuprous oxide is not precipitated. This property is made use of in the estimation of sugar by Pavy's method.

Try Trommer's test with a little glucose solution to which ammonium chloride has been added.

(iii) **Barfoed's test.** Add to some Barfoed's reagent (acetic acid solution of cupric acetate) some sugar solution, drop by drop, keeping it boiling until red cuprous oxide is precipitated.

This test is only given by glucose (fructose) and monosaccharides and not by maltose, lactose and disaccharides. It is chiefly used for distinguishing between maltose and glucose.

The Barfoed's reagent must be freshly prepared, otherwise maltose and lactose will reduce it.

i.e by the monosaccharides and not the disaccharides.

(*c*) Bismuth.

Boettger's test. To some of the sugar solution add a few crystals of bismuth subnitrate and twice the quantity of sodium carbonate. The bismuth hydrate first formed becomes reduced to metallic bismuth on boiling for some time, and the precipitate becomes grey or black in colour.

It is carried out better as modified by Nylander (cf. the Trommer and Fehling tests).

Nylander's test. Boil for 2-5 minutes about 1 part of Nylander's reagent (100 gm. NaOH sp. gr. 1·12, 4 gm. NaKTart, 2 gm. bismuth subnitrate) with 5 parts of the sugar solution. On cooling a black precipitate settles out.

These reactions are particularly useful for detecting small quantities of glucose in urine. The uric acid in urine also reduces Fehling's solution, but not Nylander's solution.

4. **Osazone test.** To a little of the sugar solution add equal quantities of phenylhydrazine and glacial acetic acid (5 to 10 drops of each) or 1 part of phenylhydrazine hydrochloride and 2 parts of sodium acetate. Place in a water bath at 100° for $\frac{1}{2}$ to 1 hour. Note when the yellow crystalline mass of phenyl glucosazone is deposited. Then cool, or better allow to cool. Filter off the crystals, examine them under a microscope and compare with fig. 13.

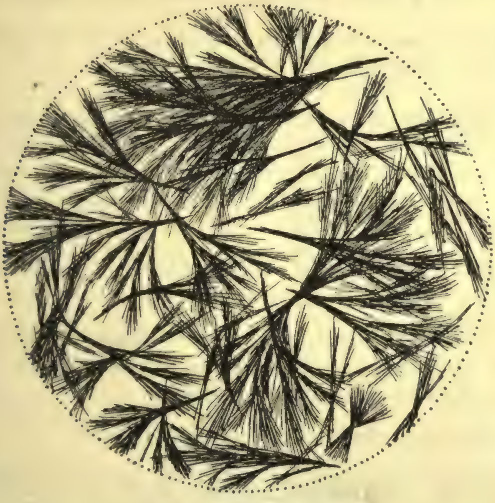


FIG. 13.—Glucosazone.

The reaction which occurs here is similar to that with aldehydes, the hydrazone being first formed. The secondary alcohol group next to the aldehyde group is then oxidized to the ketone group, which also combines with phenylhydrazine and an osazone is formed, thus :—

$$C_4H_9O_4(CHOH)CHO + H_2N.NH.C_6H_5 = C_4H_9O_4(CHOH)CH:N.NH.C_6H_5 + H_2O$$

$$\begin{gathered}C_4H_9O_4(CHOH)CH:N.NHC_6H_5 + H_2N.NH.C_6H_5 = \\ C_4H_9O_4(CO)CH:N.NHC_6H_5 + H_2N.C_6H_5 + NH_3\end{gathered}$$

$$\begin{gathered}C_4H_9O_4(CO)CH:N.NH.C_6H_5 + H_2N.NH.C_6H_5 = \\ C_4H_9O_4(C:N.NHC_6H_5)CH:N.NHC_6H_5 + H_2O\end{gathered}$$

5. **Action of Hydrochloric Acid.** Add an equal quantity of concentrated hydrochloric acid to the sugar solution and heat for some time. The solution becomes brown and "humus" substances separate out.

6. **Fermentation test.** Rub up a little fresh yeast with some glucose solution. Place the mixture in a test tube in a water bath at 25° C. Observe that after a short time bubbles of carbon dioxide rise to the surface. If left for twenty-four hours most of the glucose will have disappeared and alcohol may be detected.

7. **Molisch's test.** Add a drop of α-naphthol solution to a small quantity of the sugar solution. Then run in to the bottom of the test tube about 2 c.c. of concentrated sulphuric acid. A violet ring appears at the point of contact, either at once or after a short time. Mix the liquids, taking care to keep cool under the water tap. The colour becomes reddish-violet. On examining with the spectroscope a band between D and E is seen, whilst the violet end is totally absorbed (cf. p. 221).

This reaction is used principally for detecting the presence of carbohydrate in proteins.

8. **Polarization.** Observe the dextro-rotation of the glucose solution in the polarimeter (p. 74).

B. FRUCTOSE (LAEVULOSE).

Perform the above reactions 1, 2, 3(b), 4, 5, 6, 9, with the fructose solution. All are positive.

Note 1. Although fructose is a ketose it nevertheless reduces metallic oxides in alkaline solution. This is due to the terminal $-CO.CH_2OH$ group which is easily oxidizable. Though acetone does not reduce metallic oxides, monohydroxyacetone $CH_3.CO.CH_2OH$ does, as it contains the above grouping.

2. Fructose gives an osazone identical with phenyl glucosazone. Compare the crystals under the microscope.

3. When boiled with concentrated hydrochloric acid the solution of fructose generally becomes red or red-brown in colour before it ultimately turns dark brown.

4. Fructose solutions are lævo-rotatory.

Special test—Seliwanoff's test. Add to a mixture of equal parts of concentrated hydrochloric acid and water a very small quantity of fructose solution and then a few crystals of resorcinol. Heat. The solution becomes red in colour and deposits a brownish-red precipitate, which dissolves in alcohol giving a red solution.

C. GALACTOSE.

The same reactions are given by galactose. It differs from glucose in having a higher dextro-rotatory power and in forming a different osazone.

D. THE PENTOSES (ARABINOSE, XYLOSE).

They possess all the ordinary reduction reactions, etc., of monosaccharides, but do not ferment with yeast. The following two reactions are characteristic of the pentoses and are not given by the other carbohydrates.

Prepare a solution of the pentoses by boiling some gum arabic solution or some straw or pine wood with equal volumes of concentrated hydrochloric acid and water for 5 to 10 minutes. Neutralize a small portion with caustic soda and test for reducing sugar.

1. Phloroglucinol Reaction.

Mix equal parts of concentrated hydrochloric acid and water and add a little phloroglucinol. Then add a little gum arabic solution (or hydrolyzed wood solution) and warm in the water bath (or very gently over flame). The solution gradually becomes cherry red in colour and a precipitate is formed. Allow to cool. Shake with amyl alcohol; this dissolves the precipitate giving a red solution. This shows an absorption band between D and E when examined with the spectroscope.

2. Orcinol Reaction.

Add a little orcinol to equal parts of concentrated hydrochloric acid and water. Then add some gum arabic solution (or hydrolyzed wood solution) and heat in the water bath. The solution becomes red, then violet, and finally blue or blue-green with the separation of a precipitate. This dissolves in amyl alcohol giving a blue-green solution, which shows an absorption band between C and D, but near D.

E. GLYCURONIC ACID.

Hydrolyze a small portion of the Indian yellow by boiling it with equal parts of concentrated hydrochloric acid and water; cool and filter off the euxanthon.

(1) Neutralize a portion of the filtrate with caustic soda and perform Fehling's test.

omit

(2) The phloroglucinol reaction and the orcinol reaction for pentoses are positive. It may therefore be mistaken for pentoses.

Glycuronic acid does not ferment.

The free acid is dextro-rotatory, but when combined it is lævo-rotatory.

REACTIONS OF THE DISACCHARIDES.

A. SUCROSE or CANE SUGAR. { Fructose. Glucose.

1. Cane sugar does not give Moore's test.

2. Cane sugar solutions do not reduce alkaline solutions of metallic oxides.

3. Cane sugar gives no osazone with phenylhydrazine and acetic acid.

4. Cane sugar after hydrolysis into glucose and fructose gives the above three reactions. This mixture is known as invert sugar.

Add a little dilute sulphuric acid to some cane sugar solution and boil for a short time. Then neutralize with soda and test with Fehling's solution. Reduction occurs.

5. Cane sugar gives Seliwanoff's reaction.

6. Cane sugar is dextro-rotatory, but after hydrolysis by acids the mixture of glucose and fructose shows lævo-rotation, due to the lævo-rotation of fructose being greater than the dextro-rotation of glucose.

In order to properly observe this the solution of cane sugar after hydrolysis should be brought to exactly the same volume as it was before hydrolysis. (A certain amount of water is lost during boiling.)

7. Cane sugar ferments with yeast, owing to its conversion into glucose and fructose by an enzyme termed invertase, which is present in yeast.

Table of Reactions of Monosaccharides and Disaccharides.

	Moore's Test.	Fehling's Test.	Barfoed's Test.	Osazone.	HCl.	Yeast.	Rotation.
Glucose -	+	+	+	+	+	+	+
Fructose-	+	+	+	+	+	+	—
Galactose	+	+	+	+	+	+	+
Sucrose -	—	—	—	—		+	
Maltose -	+	+	—	+		+	
Lactose -	+	+		+		—	

X X

B. **MALTOSE** $\left\{\begin{array}{l}\text{Glucose}\\ \text{Glucose}\end{array}\right.$ and C. **LACTOSE** $\left\{\begin{array}{l}\text{Galactose.}\\ \text{Glucose.}\end{array}\right.$

1. They give Moore's test.

2. They reduce metallic oxides in alkaline solution.

3. They form osazones with phenylhydrazine.

Note that maltosazone and lactosazone are not precipitated whilst the solutions are hot, but are only deposited when the solution after heating for half an hour is allowed to cool.

Examine these osazones under the microscope and notice how they differ from each other and also from glucosazone. Compare the specimens with figs. 14 and 15.

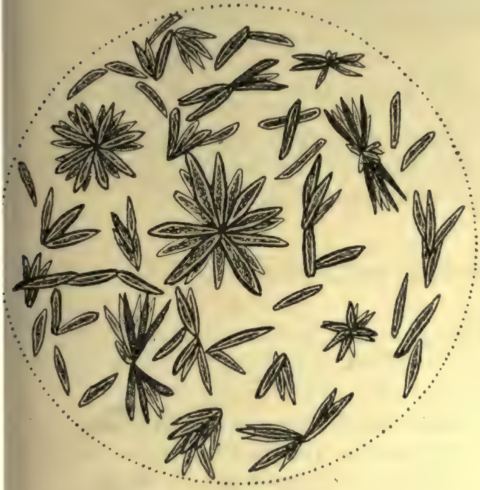


FIG. 14.—Maltosazone.

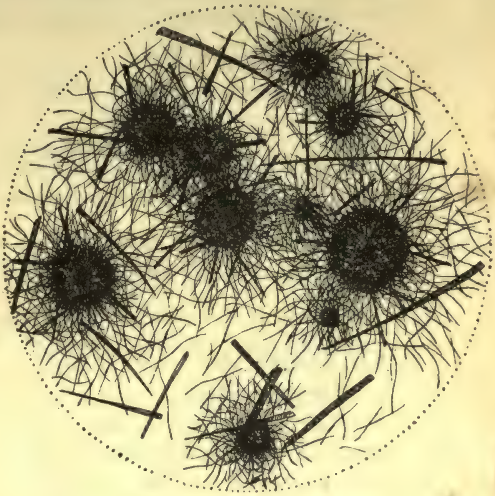


FIG. 15.—Lactosazone.

4. After hydrolysis by acids their power of reducing Fehling's solution increases. This fact will be studied more closely under "Estimation".

5. Lactose, when hydrolyzed, rotates the plane of polarized light, more. Maltose, when hydrolyzed, rotates the plane of polarized light, less. See specific rotation values (p. 78).

6. Maltose is fermented by yeast. Lactose is not fermented by ordinary yeast.

REACTIONS OF THE POLYSACCHARIDES.

A. STARCH.

Starch is a white powder insoluble in cold water. It swells up in hot water and forms on boiling an opalescent solution. Prepare a solution by mixing 1 gm. of starch with water to form a paste and pouring this into 100 c.c. of boiling water.

1. Action of Alcohol.

Alcohol precipitates starch from solution.

2. Action of Iodine.

To some of the cool solution add a few drops of iodine solution. A dark blue colour appears. Divide the solution into two halves. To one half add 1 to 2 drops of caustic soda. The colour disappears; neutralize with dilute hydrochloric acid, the colour reappears. Heat the other half. The colour disappears, but appears again on cooling.

3. Fehling's Test.

Heat another portion of the solution with a little Fehling's solution. There is no reduction.

Hydrolyze a portion of the starch solution by boiling with a little dilute sulphuric acid for a few minutes, neutralize and test with Fehling's solution. Reduction occurs due to the conversion of starch into glucose.

4. Basic Lead Acetate.

Starch solutions are precipitated by basic lead acetate.

5. Starch is not fermented by yeast.

B. GLYCOGEN.

Preparation.

Quickly remove the liver from a rabbit which has been fed five to six hours previously with carrots and killed by bleeding, and the liver washed free from blood by passing a stream of salt solution through it. Break it up into small pieces and throw the pieces into a basin of boiling water acidified with dilute acetic acid. The proteins are thus coagulated and the enzyme which converts glycogen into glucose is destroyed. Then grind up the liver in a mortar as small as possible and extract the pieces with boiling water. Combine the extracts, filter and test the opalescent solution by the following reactions :—

Reactions.

Glycogen is precipitated as a flocculent white mass on adding rather more than an equal volume of alcohol.

The opalescent solution gives with iodine a reddish-brown coloration, which disappears on heating and reappears on cooling.

The solution, if free from glucose, does not reduce Fehling's solution. On boiling the solution with acid, glucose is formed by hydrolysis and the solution on neutralization will reduce Fehling's solution.

Glycogen is precipitated by basic lead acetate solution (distinction from dextrin).

It is not fermented by yeast.

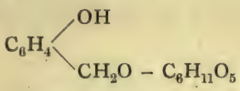
C. D[illegible]RIN.

Dextrin is obtained by the hydrolysis of starch: it is a yellow-brown powder soluble in cold water, giving a clear solution. Note the peculiar smell.

2. Alcohol precipitates it from solution.
3. Iodine gives a reddish-brown coloration; this disappears on heating and reappears on cooling.
4. Dextrin may reduce Fehling's solution very slightly. On hydrolysis by acids and neutralization Fehling's solution is strongly reduced.
5. It is not precipitated by basic lead acetate solution. (Cf. glycogen.)
6. It is not fermented by yeast.

REACTIONS OF THE GLUCOSIDES.

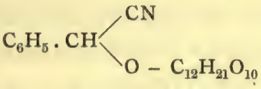
A. SALICIN.


$C_6H_4 \begin{cases} OH \\ CH_2O - C_6H_{11}O_5 \end{cases}$

(1) Salicin does not reduce Fehling's solution.

(2) Heat a little salicin solution with dilute sulphuric acid. Salicylic alcohol and glucose are formed by hydrolysis (compare polysaccharides). Neutralize with caustic soda, and test for reducing sugar by Fehling's test.

B. AMYGDALIN.


$C_6H_5 . CH \begin{cases} CN \\ O - C_{12}H_{21}O_{10} \end{cases}$

(1) A solution of amygdalin does not reduce Fehling's solution.

(2) Hydrolyze a portion of the amygdalin solution by boiling with dilute sulphuric acid. Note the smell of hydrogen cyanide. Neutralize with caustic soda and observe reduction of Fehling's solution.

Table of Reactions of Polysaccharides and Glucosides.

	Water.	Alcohol.	Iodine.	Fehling's Solution.		Basic Lead Acetate.	Yeast.
				Before hydrolysis.	After hydrolysis.		
Starch							
Glycogen -							
Dextrin -							
Salicin -							
Amygdalin							

ESTIMATION OF CARBOHYDRATES.

Three of the properties of the carbohydrates can be made use of for their estimation :—

A. Their optical activity by means of the polarimeter.

B. Their aldehydic character by reduction of cupric salts, (*a*) by Fehling's method, (*b*) by Pavy's method, (*c*) gravimetric.

C. Their fermentation by yeast.

Of these only A and B are commonly employed.

A. ESTIMATION BY MEANS OF THE POLARIMETER.

1. The Polarimeter.

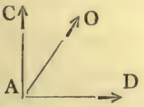
In an ordinary ray of light the vibrations of the waves take place in all planes perpendicular to the direction of its propagation. When such a ray of light is passed through a crystal of Iceland- or calc-spar and an object is observed through the crystal two images are seen. The ray of light has been split into two rays, one of which has been more refracted than the other. The more refracted or ordinary ray travels through the crystal just as it would travel through glass and obeys the laws of refraction. The less refracted or extraordinary ray, does not obey the ordinary laws of refraction, and it shows a movable image when the crystal is rotated. Both of these rays in their passage through the crystal have been polarized: i.e. *only waves in one plane* are transmitted.

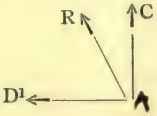
By employing a rhombohedron of Iceland-spar, cutting it across through its obtuse angles, polishing the cut surfaces and cementing together these cut surfaces with Canada balsam and blackening the longer sides a prism is obtained. On passing light through this prism the ordinary ray is totally reflected by the cut surfaces and absorbed by the blackened side whilst the extraordinary ray passes through and emerges in a direction parallel to the source of the light. Such a prism is termed, after its discoverer, a Nicol prism.

In the polarimeter two Nicol prisms mounted parallel to one another are employed. The first is fixed, the second is capable of being rotated. Light is passed through the first prism (the polarizer) and reaches the second prism (the analyser). If this second prism is exactly parallel to the first the beam of light will also pass through it; if it is not exactly parallel but inclined at an angle less light will pass through it; if the second prism is at right angles to the first the light is entirely cut off.

By interposing between the prisms set parallel to one another a solution of an optically active substance, the amount of the light is diminished, but it can be brought to its original intensity by rotating the analysing prism. The amount of rotation necessary to effect this corresponds with the power of rotation of the solution. As the analysing prism is mounted on a graduated circle, the number of degrees rotated can be measured. This is the rotatory power of the solution.

The determination of equal illumination of light in such an instrument before and after its passage through an optically active solution is very difficult and the readings are erroneous. Several devices have been adopted to overcome this difficulty, the simplest being that of Laurent. Laurent placed behind the polarizer a quartz plate of special thickness and of such a size that it covered half the field. This quartz plate divides the ray of light passing through it into two rays one of which is retarded by half a wave length and therefore reversed in direction, whilst the other is unaffected. The resultant ray on emergence formed by their fusion will be vibrating in a plane at an angle to the original plane, i.e. the polarized light passing through the quartz plate is rotated through a certain angle. Thus, if AO be the original plane before passage through the quartz plate, it is resolved into AC and AD. Supposing AD is retarded and reversed, then the components AC and AD¹ will form the resultant plane AR. This is at an angle to AO.



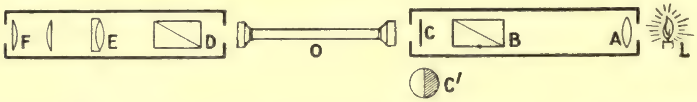


Two beams of polarized light at an angle to one another will therefore reach the analyser. If the analyser be set parallel to the beam arriving from the uncovered portion this half of the field will appear light and the other half will appear dark. If it be set parallel to the beam coming from the covered portion this half of the field will appear light and the other half dark. By adjusting the analyser a position will be found where the two halves will appear equally illuminat[illegible] This position is the zero point.

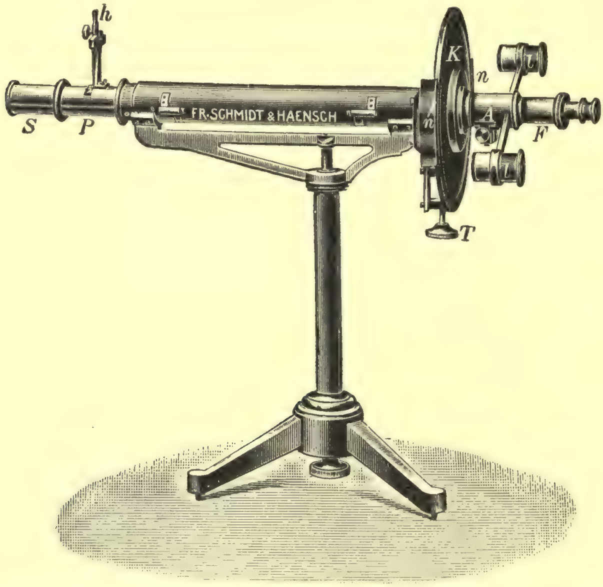
In determinations with such a polarimeter monochromatic sodium light must be used, and in this case a cell containing potassium bichromate is introduced in front of the polarizer to cut off blue rays.

In other polarimeters such as Lippich's, monochromatic light of any colour may be employed and green light from a mercury lamp is frequently used.

A polarimeter will thus consist of a bichromate cell, a polarizing prism, a quartz plate over half the field, a trough to take the solution to be examined, an analysing prism mounted in a movable circle graduated in degrees. There is in addition a telescope to focus the edge of the quartz plate and a double vernier on each side of the circle in which the analyser is mounted. This vernier is fixed and graduated in fractions of a degree or in minutes.



L = source of light. A = lens to render rays of light parallel. B = polarising prism. C, C′ = quartz plate. O = observation tube. D = analysing prism. E, F = telescope.
FIG. 16. (From Findlay's "Practical Physical Chemistry".)



At S, lens and bichromate cell. At P, polarising prism. At *h*, lever to rotate polarising prism. At A, analysing prism which can be rotated by a screw. At F, telescope with eye-piece. K = graduated scale. *n*, *n′* = fixed verniers. T = screw for rotating graduated scale. *l* = magnifying lens to read scale and verniers.
FIG. 17.

At the point of equal illumination of the two halves of the field the zero of the circular scale coincides, or very nearly coincides, with the zero of the vernier. The exact position must be determined. When an optically active substance is placed between the prisms and equal illumination of the two fields restored, the circular scale will have moved in a clockwise direction (= dextro-rotation) or in a counter clockwise direction (= lævo-rotation) from the vernier. The distance apart of the two zeros measured on the circular scale gives the amount of rotation in degrees; the fraction or minutes more is given by the vernier scale.

Several observations of the zero point of the instrument and then several of the solution must always be made. The mean of each is taken and the difference gives the rotation.

The solution of the substance is placed in a special observation tube. These tubes are 0·5, 1, 2, 2·2, etc., decimetres in length, and consist of a glass tube of the exact length which is closed by glass cover glasses held in place by a brass cap and rubber washer.

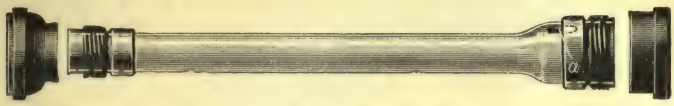


FIG. 18.

These tubes are thoroughly dried by pushing a plug of filter paper through them or thoroughly washed by rinsing several times with the solution under examination. The cover glasses must be dry and without serious scratches. One end of the tube is closed by a cover glass, brass cap and washer, and the solution is filtered into it at the other end until a meniscus just projects above the opening. A short time is given to allow air-bubbles to rise. The other cover glass is then pushed horizontally over the end of the tube driving over the excess of liquid in such a way that it exactly covers the end and leaves no air-bubbles underneath it and no liquid on its upper surface. The brass cap and washer is then screwed down over it. The brass caps must not press too tightly on the glass covers.

2. Estimation.

As the rotatory powers of all the common optically active compounds have now been determined, use can be made of these values to determine the strength of an unknown solution. These values are expressed as specific rotatory power, i.e. the rotatory of 1 gm. of substance in 1 c.c. of liquid examined in a layer one decimetre (10 cm.) long, i.e. it is the rotatory power of a 100 per cent. solution. This has not actually been carried out, but it has been calculated from the rotations of exactly known strengths of solution. The symbol $[\alpha]_D$ is used to express this value, the D standing for sodium light.

The following are the values for the principal sugars :—

Glucose = + 52·5°
Fructose = − 93·8°
Galactose = + 82°
Lactose = + 52·4°
Maltose = + 137·7°
Sucrose = + 66°

The strength of the solution is then given by the formula :—

$$[\alpha]_D = \pm \frac{\alpha \times 100}{c \times e}$$

in which $[\alpha]_D$ = specific rotation.
α = observed rotation.
c = concentration.
e = length of tube in decimetres.

Determine the percentage of glucose, fructose, and lactose in the solutions given.

B (i). ESTIMATION BY FEHLING'S METHOD.

Measure out with a pipette 10 c.c. of *freshly prepared* Fehling's solution into a porcelain basin, dilute it with about 40 c.c. of water and raise to the boiling-point.

Then from a burette run in 1 c.c. at a time of the sugar solution, keeping the Fehling solution gently boiling the whole time and *allowing the reduction to complete itself before adding a fresh quantity of the sugar solution.*

Note when the blue colour is entirely destroyed. The solution may become slightly yellow, due to the action of the alkali of the Fehling solution on the excess of the sugar. This rough titration gives a preliminary idea of how much sugar is present.

If less than 5 c.c. of glucose solution have been used, the sugar solution must be diluted until between 5 c.c. and 10 c.c. would be required to decolorize the 10 c.c. of Fehling solution. It is best to have as nearly as possible 10 c.c. dilute sugar solution = 10 c.c. Fehling solution, i.e. the solution must contain 0·5 to 1 per cent. glucose. If more, a known volume of solution must be concentrated to a smaller volume: say 100 c.c. to 20 c.c. Suppose 3·0 c.c. of the sugar solution were sufficient, Then 3 c.c. must be made up to 10 c.c.

Dilute the sugar solution by measuring out 30 c.c. with a pipette into either a 100 c.c. measuring flask and then filling up exactly to the mark with water, or into a dry flask and then with a clean pipette adding as much water as may be required for the above dilution.

Thoroughly rinse out the burette with water and then with a little of the diluted sugar solution, before putting this into it for the final titrations.

Now repeat the titration with 10 c.c. Fehling solution diluted as before, but run in at once a little less than the amount of sugar solution required to decolorize the Fehling solution entirely (say 8 c.c.). Then add cautiously 0·1 to 0·2 c.c. at a time until the decolorization is complete.

Repeat the process again, running in practically all the glucose solution necessary at one time and then completing, if necessary, with 0·1 c.c. at a time. Make, at least, two determinations of the last stage and take the mean. Suppose 10·1 c.c. were insufficient, but 10·3 c.c. too much, then 10·2 c.c. is the value.

The great difficulty is in the determination of the end point, i.e. when the blue colour is completely discharged. It is made more easy by diluting with dilute caustic soda instead of with water. This causes the cuprous oxide to settle, or, if in very large excess, keeps it in solution. (*Lavalle.*)

The difficulty is best surmounted by making use of *Ling's indicator.* This consists essentially of a solution of ferrous thiocyanate. When treated with a cupric salt, the ferrous salt is oxidized to ferric, which has a bright red colour. The indicator is made up as follows:—

1·5 gm. ammonium thiocyanate } are dissolved in 10 c.c. water
1·0 gm. ferrous ammonium sulphate } at about 45° and immediately cooled.

5 c.c. concentrated hydrochloric acid are then added.

The solution has usually a brownish-red colour: this is removed by adding a small quantity of zinc dust.

In practice, the estimation is carried out in a small flask in preference to a basin, and when nearing the end point at which the blue colour is discharged drops are removed from the solution and placed against one of a series of drops of indicator upon a glass plate on a white surface. The reduction of the Fehling solution is complete when a red coloration is no longer produced.

Knowing the dilution, the amount of sugar in the original solution can be calculated :—

$$10 \text{ c.c. Fehling solution} = 0{\cdot}05 \text{ gm. glucose.}$$

$$\therefore\ 10{\cdot}2 \text{ c.c diluted sugar solution} = 0{\cdot}05 \text{ gm. glucose.}$$

$$\text{Now } 100 \text{ c.c. diluted sugar solution contain } 30 \text{ c.c. original sugar solution.}$$

$$\therefore\ 10{\cdot}2 \text{ c.c. diluted sugar solution contain } \frac{30 \times 10{\cdot}2}{100} \text{ original sugar solution.}$$

$$\therefore\ \frac{30 \times 10{\cdot}2}{100} \text{ c.c. original sugar solution} = 0{\cdot}05 \text{ gm. glucose.}$$

$$\therefore\ 100 \text{ c.c. original sugar solution} = \frac{100 \times 100 \times 0{\cdot}05}{30 \times 10{\cdot}2}$$

$$= 1{\cdot}6 \text{ per cent.}$$

B (ii). ESTIMATION BY PAVY'S METHOD.

In order to overcome the difficulty with the precipitate of cuprous oxide Pavy has modified the Fehling method by carrying out the process in ammoniacal solution, which keeps the cuprous oxide in solution (cf. p. 66).

Measure out 50 c.c. of Pavy solution with a burette into a 200 c.c. conical flask, and add an equal volume of water. Cork the flask with a doubly-bored stopper; into one opening place the end of the burette, seeing that all the air is displaced, and into the other place a bent tube to carry off the steam and ammonia. Pass this by rubber tubing into a beaker of water containing a little acid. Boil gently to drive out the air and then run in the sugar solution *slowly* (0·5 to 1 c.c. at a time). Allow time between each addition for the reduction to take place, which is slower than with the Fehling solution. Continue until the blue colour is discharged. If the sugar solution be run in too rapidly, acid will be sucked back into the flask owing to the cooling of the liquid.

Just as in Fehling's method, the sugar solution must be diluted so that about 10 c.c. = 50 c.c. Pavy solution.

The estimations are made by running in rather less than the amount required and finishing off more slowly; the sugar solution must be run in at such a rate that the Pavy solution is kept gently boiling all the time. The whole process must be completed *within three minutes*, otherwise the ammonia is all evolved before the titration is completed and cuprous oxide is deposited. The boiling must not be interrupted.

Dilute sugar solutions and strong sugar solutions both containing the same amount of glucose give different readings. This is because the ammonia is diluted by the dilute solution and hence the process is only accurate when 10 c.c. sugar solution = 50 c.c. Pavy solution. The varying factor is the evolution of the ammonia.

For proper accuracy the process should be repeated with a fresh portion of Pavy solution running in slowly about 0·5 c.c. less than that just found. If this is sufficient a still smaller quantity must be run in until the exact minimal amount is found to completely reduce the Pavy solution.

Calculate the percentage of sugar

10 c.c. Pavy solution = 0·005 gm. glucose.

ESTIMATION OF DISACCHARIDES.

A. Cane Sugar.

Cane sugar is estimated by taking the reducing power after hydrolysis by acid; fructose and glucose have the same reducing power.

Hydrolyze a known volume of the cane sugar solution (say 40 c.c.) by warming it on the water bath for 5 to 10 minutes with 2 c.c. of dilute hydrochloric acid. Cool, neutralize with caustic soda and make up to a definite volume in a measuring flask (say 50 c.c.) rinsing out the flask with the water necessary to make up the 50 c.c. Then place the solution in a burette and run it into Fehling's solution or Pavy's solution as previously described. The sugar solution should be of a strength so that 10 c.c. = 10 c.c. Fehling or 50 c.c. Pavy solution.

Calculate the percentage of cane sugar :—

$$\underbrace{C_{12}H_{22}O_{11}}_{342} + H_2O = \underbrace{2C_6H_{12}O_6}_{360}$$

$$0{\cdot}047 \qquad\qquad 0{\cdot}05 = 10 \text{ c.c. Fehling solution.}$$

B. Lactose and Maltose.

Lactose and maltose are estimated in the same way as glucose with Fehling solution, but their reducing power is less than that of glucose. Consequently, a factor has to be employed in order to obtain the true value. This is obtained by determining the reducing power before and after hydrolyzing them into monosaccharides by acid.

Determine the percentage of lactose in the given solution.

Take a definite volume of the lactose solution, and dilute it, as previously done for glucose, with water so that 10 c.c. reduce 10 c.c. Fehling solution (or 50 c.c. Pavy solution). Then take the reducing power exactly.

Hydrolyze an exactly equal quantity of the lactose solution by boiling it for 10 minutes in a flask with about one-tenth of its volume of dilute sulphuric acid. Cool, neutralize with soda and make up to the same volume as the non-hydrolyzed lactose solution with water.

It is best to make up the solutions in a measuring flask, e.g. 25 c.c. lactose solution are diluted to 100 c.c.; 25 c.c. lactose solution are hydrolyzed by acid, neutralized and washed into the 100 c.c. measuring flask.

Take the reducing power of the hydrolyzed lactose solution exactly.

$$\text{The factor is } \frac{\text{reducing power of lactose in c.c.}}{\text{reducing power of hydrolyzed lactose in c.c.}} = \frac{10}{7}.$$

The reducing powers of gluctose : lactose : maltose are as 1 : 0·71 : 0·63.

Calculate the percentage of lactose :—

$$x \text{ c.c.} = 10 \text{ c.c. Fehling solution.}$$

$$\therefore x \times \frac{7}{10} \text{ c.c.} = 0{\cdot}05 \text{ gm. lactose.}$$

$$\text{or } x \text{ c.c.} = 0{\cdot}05 \times \frac{10}{7} \text{ gm. lactose.}$$

$$\text{Hence } 100 \text{ c.c.} = 0{\cdot}05 \times \frac{10}{7} \times \frac{100}{x} \text{ gm. lactose.}$$

ESTIMATION OF POLYSACCHARIDES.

All polysaccharides are estimated in the same way as cane sugar, i.e. by taking the reducing power of a known weight or volume of the solution after hydrolysis by acid, i.e. in terms of glucose :—

$$C_6H_{10}O_5 + H_2O = C_6H_{12}O_6.$$

C. ESTIMATION BY FERMENTATION.

Sugar is sometimes estimated by fermentation with yeast in an Einhorn fermentation tube. This is a U-shaped tube one limb of which is closed and the other expanded into a bulb (fig. 19).

The closed limb of these tubes is filled with the sugar solution to which some yeast has been added. Mercury is placed at the bend. The carbon dioxide evolved collects in the closed limb and drives down the solution into the other and wider limb. The narrow limb is graduated in percentages of glucose, so that the amount of sugar can be directly read off.

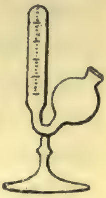


FIG. 19.

Examine the Einhorn fermentation tubes in which various strengths of glucose solution have been fermented with yeast.

Another method of estimation is by taking the specific gravity of the solution before and after fermentation.

The fermentation method is not very accurate and is not often used for estimating sugar.

ACTION OF ENZYMES.

The two classes of compounds, the fats and carbohydrates, make up the main portion of the non-nitrogenous organic material of both plants and animals, and are concerned intimately with the functional activity of the organism. What is their origin, what changes do they undergo in nature, and how are these changes brought about?

The carbohydrates arise in nature from the carbon dioxide in the atmosphere and water, and it was suggested in 1870 by Baeyer that formaldehyde was the first product, the carbon dioxide being reduced with evolution of oxygen:—

$$CO_2 + H_2O = H{\cdot}CHO + O_2.$$

This stage in the synthesis remained hypothetical for many years, but in 1902 Usher and Priestley succeeded in showing that in the presence of sunlight the chlorophyll of the green plant could effect this change, and confirmation of the reaction was given by Schryver in 1909.

As soon as it is formed the formaldehyde is converted into carbohydrate (by aldol condensation?) by the protoplasm (enzymes) of the chloroplasts. According to Sachs the first visible carbohydrate to be formed is starch; according to Horace Brown and Escombe cane sugar is the first product. Whether the intermediate stage of glucose or fructose occurs has not been definitely ascertained. The process is therefore as follows:—

$$6CH_2O = C_6H_{12}O_6\ ?$$

$$2C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11}$$

$$nC_6H_{12}O_6 - (n\text{-}1)H_2O = n(C_6H_{10}O_5).$$

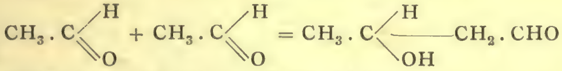
Mannose, glucose, and fructose can be converted into one another by the action of alkalies. The formation of galactose is supposed to occur in a similar manner.

The converse stages in the process are better known. The complex polysaccharide or disaccharide is hydrolyzed into its simple monosaccharide units. The monosaccharide is converted into carbon dioxide and alcohol, lactic acid and other products.

In both plants and animals fats can be formed from carbohydrate. Sugar is present in the cell sap of plants, but not fat; unripe seeds and nuts contain sugar and no fat, the ripe seed contains large quantities of fat. Most of the fat in animals is derived directly from the fat in the food, but fat can be built up by animals from carbohydrates as was shown by the experiments of Lawes and Gilbert on the fattening of farm stock.

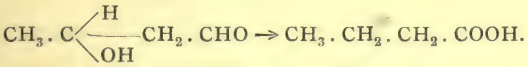
A direct condensation of three molecules of glucose would yield a chain of eighteen carbon atoms, and if reduction of the hydroxyl groups occurred at the same time stearic acid might be formed. Such a transformation will not lead to the formation of the other fatty acids with fewer carbon atoms.

In all probability the transformation of carbohydrate into fatty acid consists firstly in the disjointing of the six carbon atom carbohydrate complex into two molecules of glyceric aldehyde. From this compound by simultaneous reduction of the β-hydroxyl group and oxidation of the terminal aldehyde group to the carboxyl group lactic acid may be formed. Lactic acid is a common resultant product of the action of bacteria on glucose, and it is also present in the animal body. Lactic acid on oxidation breaks down at the α carbon atom giving acetaldehyde and carbon dioxide. Two molecules of acetaldehyde undergoing the aldol condensation will give a compound with four carbon atoms:—



$$CH_3 . C\begin{smallmatrix}\diagup H\\ \diagdown\!\diagdown O\end{smallmatrix} + CH_3 . C\begin{smallmatrix}\diagup H\\ \diagdown\!\diagdown O\end{smallmatrix} = CH_3 . C\begin{smallmatrix}\diagup H\\ \diagdown OH\end{smallmatrix}\!\!-\!\!-\!\!-CH_2 . CHO$$

and if simultaneous reduction of the alcohol group and oxidation of the aldehyde group, i.e. transfer of the γ oxygen atom to the aldehyde group occurs, butyric acid will be formed:—



$$CH_3 . C\begin{smallmatrix}\diagup H\\ \diagdown OH\end{smallmatrix}\!\!-\!\!-\!\!-CH_2 . CHO \rightarrow CH_3 . CH_2 . CH_2 . COOH.$$

The condensation of more than two molecules of acetaldehyde in a similar manner would give chains with 6, 8, 10, 12, 14, 16, 18 carbon atoms. Such chains with an even number of carbon atoms are present in the natural fatty acids; fatty acids with an odd number of carbon atoms are not found in nature.

Glycerol, the other constituent of fats, no doubt arises from the further reduction of glyceric aldehyde. The formation of the ester, fat, is then effected.

The hydrolysis of the fat into its constituents is the first stage in the converse change. This hydrolysis always precedes any transference of fat from one tissue to another. The further changes are effected by oxidation. Glycerol is readily oxidized into carbon dioxide and water.

There is a great deal of evidence to show that the first change in the fatty acid is one of desaturation, i.e. the introduction of unsaturated bonds into the chain, and that this is effected in animals by the liver. At these points the long carbon chain is weakened, and it splits into two molecules, one a fatty and the other a dibasic acid, each of them with fewer carbon atoms.

The further oxidation of these molecules then occurs. The fatty acid breaks down chiefly at the β-carbon atom : the long chain will thus lose two carbon atoms at a time. β-oxybutyric acid is a stage in the oxidation of butyric acid in the organism : on further oxidation it gives acetyl acetic acid ; fat is therefore an origin of this oxy acid. The dibasic acid probably loses carbon dioxide and passes into a fatty acid ; the fatty acid is then further oxidized as above.

The hydrolysis and decomposition of these compounds is effected by the reagents grouped under the term enzymes which are present in the neutral or faintly alkaline tissues of animals and plants.

Enzymes are either excreted in the juices by definite cells or glands in the organism, e.g. saliva, pancreatic juice: that is, they act outside the cells which produce them. Or they are not excreted: that is, they act inside the cell envelope. For purposes of investigation these can be obtained by crushing the cell envelope and liberating the cell contents. A pure preparation of an enzyme has not as yet been obtained either from the excreted juices or from the cell contents. Their chemical nature is unknown and they are recognized only by their activity.

Enzymes differ from acids and alkalies in the following particulars:—

1. They act in very small concentration, i.e. as catalysts.
2. They are destroyed at high temperatures. Their action is most rapid at or slightly above the body temperature (37° C.) and is nil at 0° C.

3. They are very sensitive to acid and alkali. Most enzymes act best in a very faintly alkaline medium: some enzymes act only in a slightly acid medium. Their action is inhibited and destroyed by dilute acids and alkalies.

4. They are specific in their action. A given change in a compound or class of compound can be effected by *only* one enzyme. Hence their large number.

Enzymes are designated by the suffix -ase, the first part of the word being composed of the name of the substance upon which the enzyme acts. The substance upon which the enzyme acts is known as the substrate or hydrolyte.

The following table gives the enzymes which act upon the carbohydrates and fats, and the products which result:—

ENZYME.	SUBSTRATE.	PRODUCT.
Diastase or Amylase.	Starch or Amylum.	Dextrin + Maltose.
	Glycogen.	,, ,,
Inulase.	Inulin.	Fructose.
Invertase or Sucrase.	Cane Sugar.	Glucose + Fructose.
	Raffinose.	Fructose + Melibiose $\left\{\begin{array}{l}\text{Glucose.}\\ \text{Galactose.}\end{array}\right.$
Lactase.	Lactose.	Glucose + Galactose.
Maltase or α-Glucase.	Maltose.	Glucose + Glucose.
	α-Glucosides.	α-Glucose.
Emulsin or β-Glucase.	β-Glucosides.	β-Glucose.
	Amygdalin.	Benzaldehyde,HCN,2 mols. Glucose.
Zymase.	Glucose. Fructose. Mannose. Galactose.	CO_2 + Alcohol.
Lipase.	Fats.	Glycerol + Fatty Acid.

I. Investigation of the Action of Diastase or Amylase.

Ptyalin + ne of Diastases

Diastase acts upon starch and glycogen giving rise to the following products : 1. Soluble starch, 2. erythrodextrin, 3. achroodextrin, 4. maltose.

Prepare some dilute saliva by rinsing out the mouth thoroughly with 20 c.c. of distilled water, warmed to 40° C., for 1 to 2 minutes. Repeat this operation 2 or 3 times and collect the washings in a beaker. Filter.

Place a series of drops of iodine solution (I in KI) upon a porcelain plate, or upon a glass plate on white paper. To one of them add a drop of starch solution ; a blue colour is formed.

Take two test tubes and into each put 5 c.c. of starch solution. Into one of them place 5 c.c. of water, and into the other 5 c.c. of dilute saliva solution. Place both tubes in the water bath at 40° C. The first tube serves as a control. Observe that the mixture in the second tube soon becomes less

blue with I ; 1 opaque. This is due to the formation of soluble starch. Take out a drop of the solution with a glass rod and apply it to the next iodine drop. A blue colour (probably mixed with a reddish-brown) will appear.

Then, at intervals of half a minute or a minute, take out drops of the same solution and apply them to the iodine drops. The blue colour will soon cease to appear at all, but will be replaced by a reddish-brown colour.

red " I. 2 This is due to the presence of erythrodextrin.

Eventually no colour will be given with the iodine solution. Achroo-

no colour " I 3 dextrin has been formed, which gives no colour with iodine ; also maltose. Finally, after 5 to 10 minutes, test the solution with Fehling's solution.

Reduces Fehling 4. Reduction will occur owing to the formation of maltose from the starch. The other solution, which has served as a control, will be found to give a blue colour with the iodine drops, and will not reduce Fehling's solution.

Effect of Temperature.

Perform the same experiment with two test tubes each containing 5 c.c. of starch solution and 5 c.c. of saliva solution, but place one of them in cold water, or better ice, and the other in the water bath at 40° C. Compare the times when the various colours are given and when no colour is given with the iodine drops. The change takes place more slowly in the tube kept at the lower temperature.

Again take 5 c.c. of starch solution, but add to it 5 c.c. of boiled saliva solution and place it in the water bath at 40° C. No action occurs as the enzyme has been destroyed by boiling.

This experiment is generally used as a control in enzyme experiments. Two experiments are carried out side by side under identical conditions, except that in the one case boiled enzyme solution is used.

Effect of Acid, Alkali, and Salt upon Diastase.

Into one test tube, place 5 c.c. of starch solution, 1 c.c. of water, and
1. 5 c.c. of saliva solution.

Into a second test tube, place 5 c.c. of starch solution, 1 c.c. of dilute
2. hydrochloric acid ($\frac{N}{10}$ or 0·4 per cent.), and 5 c.c. of saliva solution.

Into a third test tube, place 5 c.c. of starch solution, 1 c.c. of dilute acetic
3. acid (0·5 per cent.), and 5 c.c. of saliva solution.

Into a fourth test tube, place 5 c.c. of starch solution, 1 c.c. of dilute alkali
4. ($\frac{N}{10}$ or 0·4 per cent.), and 5 c.c. of saliva solution.

Into a fifth test tube, place 5 c.c. of starch solution, 1 c.c. of sodium
5. chloride solution (1 per cent.), and 5 c.c. of saliva solution.

Place the five tubes in the water bath at 40° C. and at intervals examine drops from each with iodine solution.

1 Hydrochloric acid completely stops the action of diastase; 2 acetic acid hinders the action, i.e. the conversion of starch into achroodextrin and maltose takes longer; 3 alkali may hasten, but if strong will stop the action. 4 A small concentration of sodium chloride hastens the action; 5 sodium chloride of a concentration of 5 per cent. will hinder the action.

Specificity of Action.

Add 5 c.c. of saliva solution to 5 c.c. of cane sugar solution and keep at 40° C. for some time. Test for reducing sugar with Fehling's solution. There is no conversion of cane sugar by diastase into glucose and fructose.

Perform the same experiment with 5 c.c. of salicin solution instead of cane sugar solution. Again there is no action (reduction of Fehling's solution).

II. Investigation of the Action of Invertase.

Invertase acts upon cane sugar giving glucose and fructose.

To 5 c.c. of the cane sugar solution in a test tube add 1 c.c. of invertase solution and place in the water bath at 40° C. for 10 to 15 minutes. At the same time place another test tube containing 5 c.c. of cane sugar solution and 1 c.c. of boiled enzyme solution in the water bath for this period of time.

Test both solutions for reducing sugar with Fehling's solution. Reduction occurs only in the first tube.

In the same way test the action of 1 c.c. of the invertase solution upon 5 c.c. of starch solution at 40° C. There is no hydrolysis of starch by invertase. If any action occurs it is due to impurity in the invertase solution, i.e. to its containing a little diastase. It is very difficult to obtain enzyme solutions which contain only one enzyme. Most cell contents contain a mixture of enzymes.

III. Investigation of the Action of Emulsin.

Add to 5 c.c. of the salicin solution 1 c.c. of emulsin solution and keep the mixture at 40° C. for 15 to 30 minutes. The salicin is converted into glucose and saligenin or salicylic alcohol. Show the presence of glucose by reduction of Fehling's solution.

Carry out a control experiment at the same time with boiled enzyme solution.

IV. Investigation of the Action of Yeast.

Glucose
[illegible]
[illegible]
[illegible]

Yeast contains a mixture of several enzymes. Its principal enzyme is zymase which acts upon the four natural hexose monosaccharides. Yeast contains also maltase and invertase. It does not contain lactase. This enzyme is only present in special yeasts such as kefir. It is owing to the presence of maltase and invertase, that yeast is able to ferment maltose and cane sugar and convert these into alcohol and carbon dioxide. Lactose is not fermented. Before alcoholic fermentation can occur hydrolysis into monosaccharide must take place.

Examine the Einhorn fermentation tubes in which have been placed 1 per cent. solutions of glucose, fructose, galactose, maltose, lactose, cane sugar, with a small quantity of yeast and kept at 37° C. for 12 hours.

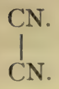
Except lactose all the sugars have been fermented: galactose is fermented more slowly than the other monosaccharides as shown by the smaller volume of carbon dioxide evolved.

V. Investigation of the Action of Lipase.

Grind up very thoroughly about 1 gramme of ground castor oil seed with 25 c.c. of chloroform water. Divide the mixture into two exactly equal portions of 10 c.c. and place them in two test tubes. Immediately boil one portion to destroy the enzyme. Then add to each 1 c.c. of dilute acetic acid and place both tubes in a water bath at 40° C. for at least half an hour. After this time add to each test tube a few drops of phenolphthalein and titrate with $\frac{N}{10}$ NaOH. The number of c.c. required to neutralize the tube with unboiled enzyme will be greater than in the tube with boiled enzyme. The oil in the seed has been hydrolyzed into fatty acid and glycerol by the enzyme lipase also present in the seed.

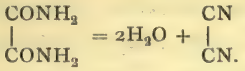
CYANOGEN COMPOUNDS.

1. **Cyanogen.**



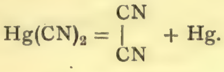
$$\begin{array}{c}CN.\\ |\\ CN.\end{array}$$

Cyanogen, as it contains only the two elements carbon and nitrogen, is the simplest carbon compound containing nitrogen. It is formed when electric sparks are passed between carbon poles in an atmosphere of nitrogen, and when oxamide (p. 108) is treated with dehydrating agents, e.g. phosphorus pentoxide:—



$$\begin{array}{c}CONH_2\\ |\\ CONH_2\end{array} = 2H_2O + \begin{array}{c}CN\\ |\\ CN.\end{array}$$

It is usually prepared by heating mercuric cyanide:—



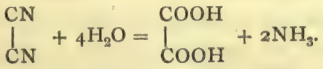
$$Hg(CN)_2 = \begin{array}{c}CN\\ |\\ CN\end{array} + Hg.$$

Cyanogen is a colourless gas with pungent smell, and it burns with a pink flame forming carbon dioxide and nitrogen. It is easily soluble in water and intensely poisonous.

Cyanogen and the other compounds of this group resemble the halogens very closely in their properties, thus when treated with alkali cyanogen is converted into alkali cyanide and cyanate:—

$$(CN)_2 + 2KOH = KCN + KCNO + H_2O.$$
$$Cl_2 + 2KOH = KCl + KClO + H_2O.$$

When boiled with acids, cyanogen is converted by hydrolysis into oxalic acid and ammonia:—



$$\begin{array}{c}CN\\ |\\ CN\end{array} + 4H_2O = \begin{array}{c}COOH\\ |\\ COOH\end{array} + 2NH_3.$$

As the compounds of this group which contain the CN radicle on hydrolysis with acids are converted into the corresponding acid they are termed nitriles: cyanogen is the nitrile of oxalic acid or oxalonitrile.

2. **Hydrogen Cyanide, or Prussic Acid.** HCN.

Hydrogen cyanide is present in numerous plants, e.g. in laurel leaves, bitter almonds, cherry and peach kernels, usually in combination with glucose and benzaldehyde as the glucoside amygdalin (p. 65) which is hydrolyzed by acids or by enzymes in the plant into its constituents. It is formed by the oxidation of glycine and other amino acids. It may thus arise in the animals and plants: the latter probably then combine it with glucose to form a glucoside, the former apparently combine it with sulphur to form sulphocyanide or thiocyanate, which is present in saliva and other secretions.

A dilute solution of hydrogen cyanide is obtained by distilling potassium ferrocyanide or a cyanide with dilute sulphuric acid:—

$$2K_4Fe(CN)_6 + 3H_2SO_4 = 6HCN + \underset{\substack{\text{Potassium} \\ \text{ferrous ferrocyanide.}}}{K_2FeFe(CN)_6} + 3K_2SO_4.$$

Pure anhydrous hydrogen cyanide is prepared by distilling potassium cyanide with strong sulphuric acid, passing the gas over solid calcium chloride and collecting the distillate in a receiver cooled by ice.

Pure hydrogen cyanide is a colourless liquid boiling at 26° C.

Hydrogen cyanide is also obtained by dehydrating ammonium formate or formamide with phosphorus pentoxide.

It is the nitrile of formic acid: its solution in water on standing or on boiling with hydrochloric acid is converted into formic acid:—

$$HCN + 2H_2O = HCOOH + NH_3.$$

Hydrogen cyanide is very similar in its properties to hydrochloric acid; its alkaline salts crystallize like sodium and potassium chloride: its silver salt is white and insoluble in water and acids, but soluble in ammonia. Silver cyanide, unlike silver chloride, is decomposed by boiling with acids forming hydrogen cyanide.

Potassium cyanide is used commercially for extracting gold from its ores and in electroplating. It is prepared by fusing potassium ferrocyanide alone, with potassium carbonate, or with sodium:—

$$K_4Fe(CN)_6 = 4KCN + N_2 + FeC_2.$$
$$K_4Fe(CN)_6 + K_2CO_3 = 5KCN + KCNO + CO_2 + Fe.$$
$$K_4Fe(CN)_6 + 2Na = 4KCN + 2NaCN + Fe.$$

Cyanides are also obtained (1) by fusing sodamide with charcoal, (2) by heating calcium carbide in the electric furnace in a current of nitrogen:—

$$\left\{\begin{array}{l} 2NH_3 + Na_2 = 2NaNH_2 + H_2. \\ 2NaNH_2 + 2C = 2NaCN + 2H_2. \end{array}\right.$$
$$CaC_2 + N_2 = Ca(CN)_2.$$

The alkali cyanides dissolve the insoluble cyanides of silver, gold, and other heavy metals forming the double cyanides, which are used in electroplating:—

$$KCN + AgCN = KAg(CN)_2.$$

These compounds are decomposed by acids into the metallic cyanide, hydrogen cyanide, and the alkali salt of the acid.

Potassium Ferrocyanide. $K_4Fe(CN)_6$.

When sodium cyanide is treated with a ferrous salt it is converted into sodium ferrocyanide (p. 15). These are complex cyanides and are not decomposed like the double cyanides by acids. If acid be added to a concentrated solution of potassium ferrocyanide, hydroferrocyanic acid is thrown down as a white precipitate. This acid forms salts with other metals, e.g. the ferric salt (Prussian blue).

Potassium ferrocyanide (yellow prussiate of potash) is prepared by fusing together protein residues, such as blood or leather, and potassium carbonate. The mass is then extracted with water and the yellow solution which results is evaporated down until it crystallizes. This compound is also prepared from the bye-products in coal gas manufacture. Hydrogen cyanide is one of the bye-products and is absorbed by iron oxide. By boiling this material with lime, calcium ferrocyanide is formed from which potassium ferrocyanide is obtained by treatment with potassium carbonate.

Potassium Ferricyanide. $K_3Fe(CN)_6$.

When chlorine or bromine is passed into a solution of potassium ferrocyanide the solution changes in colour from yellow to red, and on evaporation red crystals of potassium ferricyanide or red prussiate of potash are obtained :—

$$2K_4Fe(CN)_6 + Cl_2 = 2K_3Fe(CN)_6 + 2KCl.$$

Potassium ferricyanide does not react with ferric salts; but with ferrous salts it gives a blue precipitate of Turnbull's blue, $Fe''_3[Fe(CN)_6]$.

Potassium ferricyanide in alkaline solution is decomposed into potassium ferrocyanide and oxygen : it acts therefore as an oxidizing agent :—

$$2K_3Fe(CN)_6 + 2KOH = 2K_4Fe(CN)_6 + H_2O + O.$$

Alkyl Cyanides—Nitriles.

When potassium cyanide is treated with an alkyl iodide (methyl, ethyl, etc., iodide) the alkyl cyanide is obtained :—

$$KCN + CH_3I = KI + CH_3CN.$$

These compounds are also formed by treating the amides of the corresponding acid with phosphorus pentoxide :—

$$CH_3CONH_2 = H_2O + CH_3CN.$$

Like hydrogen cyanide they are hydrolyzed by acids into the corresponding acid :—

$$CH_3CN + 2H_2O = CH_3COOH + NH_3.$$

On reduction they are converted into amines :—

$$CH_3CN + 2H_2 = CH_3.CH_2.NH_2.$$

The lower members of the series are liquids with peculiar smell, more or less soluble in water; the higher members are solids.

Alkyl Isocyanides.

If silver cyanide be treated with alkyl iodides isocyanides, compounds isomeric with the above, are formed :—

$$CH_3I + AgCN = CH_3NC + AgI.$$

It would thus appear that silver cyanide had a different structure to potassium cyanide or that in the reaction a rearrangement occurred.

These compounds are also formed by heating primary amines (p. 37) with chloroform and potash :—

$$CH_3NH_2 + CHCl_3 + 3KOH = CH_3NC + 3KCl + 3H_2O.$$

They are liquids with an abominable smell : on hydrolysis they give the corresponding amine and formic acid :—

$$CH_3NC + 2H_2O = CH_3NH_2 + HCOOH.$$

Cyanogen Chloride. ClCN.

If mercuric cyanide, potassium cyanide, or hydrogen cyanide be treated with chlorine, cyanogen chloride is obtained :—

$$KCN + Cl_2 = ClCN + KCl.$$

This compound, which is a liquid, polymerizes on standing into solid cyanuric chloride $Cl_3C_3N_3$. Potash converts liquid cyanogen chloride into potassium cyanate, and solid cyanuric chloride into potassium cyanurate :—

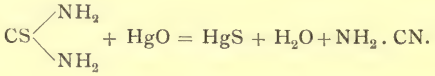
$$ClCN + 2KOH = KCNO + KCl + H_2O.$$
$$Cl_3C_3N_3 + 6KOH = K_3C_3N_3O_3 + 3KCl + 3H_2O.$$

Cyanamide. $NH_2.CN$.

When cyanogen chloride is passed into ammonia, cyanamide is formed :—

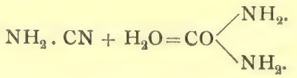
$$ClCN + NH_3 = NH_2.CN + HCl.$$

This compound is most readily prepared by the action of mercuric oxide on thiourea :—



$$CS\begin{cases}NH_2\\NH_2\end{cases} + HgO = HgS + H_2O + NH_2.CN.$$

Cyanamide is a hygroscopic crystalline substance melting at 40° C. It forms salts with strong acids and also with bases. The calcium salt is frequently employed as an artificial manure, most probably as it is readily hydrolyzed into urea :—



$$NH_2.CN + H_2O = CO\begin{cases}NH_2.\\NH_2.\end{cases}$$

Cyanamide is used in the synthesis of creatine and arginine (p. 120). These compounds behave like cyanamide in giving urea on hydrolysis.

Cyanic Acid (HOCN) **and Cyanates. Cyanuric Acid.** $H_3C_3N_3O_3$.

On heating potassium cyanide with red lead or other easily oxidizable substances, it absorbs oxygen and is converted into potassium cyanate :—

$$KCN + O = KOCN.$$

Potassium cyanate resembles potassium chlorate: its aqueous solution is unstable and is rapidly hydrolyzed into ammonia and potassium carbonate.

Ammonium cyanate is still more unstable, and on heating its solution it is converted into urea (p. 110).

The metallic cyanates are insoluble precipitates formed on adding metallic salts to potassium cyanate solution.

Cyanic acid is formed by distilling cyanuric acid: this is a still more unstable substance decomposing with explosive violence :—

$$HOCN + H_2O = CO_2 + NH_3.$$

Cyanuric acid is formed when urea is heated :—

$$3CO(NH_2)_2 = H_3C_3N_3O_3 + 3NH_3.$$

Alkyl Isocyanates and Alkyl Cyanates.

If silver cyanate be treated with an alkyl iodide, alkyl isocyanate is formed :—

$$AgNCO + CH_3I = CH_3NCO + AgI.$$

These compounds are also obtained when potassium cyanate is distilled with alkyl potassium sulphate :—

$$KNCO + CH_3 . OSO_2 . OK = CH_3 . NCO + KO . SO_2 . OK.$$

The alkyl isocyanates are liquids with a strong and suffocating odour. They polymerize on standing forming isocyanuric esters. Their structure is shown by their conversion into amines by the action of potash :—

$$CH_3 . NCO + H_2O = CH_3 . NH_2 + CO_2.$$

The alkyl cyanates are obtained by the action of cyanogen chloride on sodium alcoholate :—

$$ClCN + NaOCH_3 = CH_3OCN + NaCl.$$

They are colourless ethereal liquids, which at once polymerize to cyanurates.

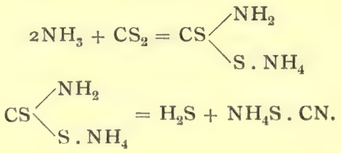
Thiocyanic Acid, Thiocyanates and Isothiocyanates.

If potassium cyanide be heated with sulphur, potassium thiocyanate is obtained :—

$$KCN + S = KSCN.$$

Thiocyanic acid results when a mineral acid is added to its salts. It is a gas which can be condensed by cold to a liquid with an acid and penetrating smell.

The ammonium salt is obtained in gasworks from the iron oxide previously mentioned. Ammonium cyanide is probably formed which is converted into thiocyanate by the action of sulphur. It can be prepared by heating together ammonia and carbon bisulphide under pressure :—



$$2NH_3 + CS_2 = CS\genfrac{}{}{0pt}{}{NH_2}{S\,.\,NH_4}$$

$$CS\genfrac{}{}{0pt}{}{NH_2}{S\,.\,NH_4} = H_2S + NH_4S\,.\,CN.$$

As previously stated thiocyanates are present in the animal body.

Alkyl thiocyanates are obtained by heating potassium thiocyanate with an alkyl iodide :—

$$KSCN + C_2H_5I = C_2H_5S\,.\,CN + KI.$$

They are oily liquids insoluble in water and have a garlic-like smell. On heating they are converted into the isomeric isothiocyanates—especially the allyl compound :—

$$C_3H_5S\,.\,CN \rightarrow C_3H_5N\,.\,CS.$$

The alkyl isothiocyanates are pungent smelling liquids and are therefore termed mustard oils. In the thiocyanates the alkyl group is joined to the sulphur atom, in the isothiocyanates to the nitrogen atom, as shown by the following reactions :—

$$CH_3SCN + 3H_2 = CH_3SH + CH_3NH_2. \qquad CH_3NCS + H_2O = CH_3\,.\,NH_2 + COS.$$

$$CH_3SCN + O \rightarrow CH_3SO_3H. \qquad CH_3NCS + 2H_2 \rightarrow CH_3\,.\,NH_2.$$

The isothiocyanates result when a primary amine and a drop of carbon bisulphide is warmed with ferric chloride and alcohol is present so as to make a homogeneous solution.

Preparation of Cyanogen.

Heat a small quantity of mercuric cyanide in a dry test tube. White fumes are given off which condense on the cooler parts of the tube. On igniting the gas which reaches the open end it will be observed to burn with the characteristic coloured flame.

Preparation and Properties of Hydrogen Cyanide.

Connect a small flask with a condenser by means of a bent tube; let the open end of the condenser dip into water in a test tube containing a drop of strong caustic soda. Now put in the flask 10 c.c. of a cold saturated solution of potassium ferrocyanide and 20 c.c. of 20 per cent. sulphuric acid. Hydrogen cyanide distils over on heating and is converted into potassium cyanide. Test for its presence by converting into Prussian blue by heating with a ferrous salt, acidifying and adding a drop of ferric chloride. Carry out the distillation in the fume cupboard.

Note.—Carbon monoxide is obtained when anhydrous potassium ferrocyanide is heated with concentrated sulphuric acid.

Boil about 20 c.c. of a 1 per cent. potassium cyanide solution. It is hydrolyzed into potassium formate and ammonia which is recognized by the blueing of red litmus paper placed in the vapours.

Heat to redness in a crucible about 1 gm. of potassium ferrocyanide. Cool and extract the mass with water. Filter. This solution contains potassium cyanide as shown by the Prussian blue reaction.

Add to 2 to 3 c.c. of 1 per cent. potassium cyanide solution a drop of dilute silver nitrate solution; the precipitate first formed dissolves on shaking owing to the formation of the double cyanide $KAg(CN)_2$. A permanent precipitate of silver cyanide is obtained on adding more silver nitrate solution. This is insoluble in dilute nitric acid.

Ferrocyanide and Ferricyanide.

Add some concentrated hydrochloric acid to a saturated solution of potassium ferrocyanide. Hydroferrocyanic acid is thrown down as a white precipitate. It turns blue on filtering, due to decomposition and oxidation.

To some potassium ferrocyanide solution add a slight excess of bromine water and then boil off the excess of bromine. If it be evaporated red crystals of ferricyanide will be obtained. On adding a drop of ferric chloride to a portion of the solution no blue colour (Prussian blue) is formed, but the solution becomes brown. If a drop of ferrous sulphate solution be added a blue precipitate (Turnbull's blue) is formed.

Render some potassium ferricyanide solution alkaline with caustic soda and add a little lead oxide. This becomes brown owing to the formation of lead peroxide PbO_2. Filter and test the filtrate with ferric chloride. A blue precipitate of Prussian blue shows the presence of potassium ferrocyanide.

Potassium Cyanate.

Heat about 1 gm. of potassium cyanide in a crucible in the fume cupboard until it melts and add to the fused mass lead oxide so long as visible reduction occurs. Extract the potassium cyanate with water. On adding sulphuric acid to the solution carbon dioxide is evolved from the decomposition of the cyanic acid. The presence of ammonia, the other product of decomposition, is shown by making the solution alkaline with soda and heating and testing the vapour with moist red litmus paper.

Potassium Thiocyanate.

Heat 10 c.c. of a 1 per cent. potassium cyanide solution for some minutes with flowers of sulphur and filter.

(*a*) On treating a portion of the filtrate with ferric chloride solution, it becomes blood-red in colour due to the formation of ferric thiocyanate.

(*b*) On adding silver nitrate solution to another portion a white curdy precipitate of silver thiocyanate is formed, which is insoluble in dilute nitric acid.

AMINES.

The amines are compounds which are derived from ammonia by the replacement of one or more hydrogen atoms by alkyl groups, e.g.

$CH_3 . NH_2$.	$(C_2H_5)_2 : NH$.	$(CH_3)_3 : N$.
Monomethylamine.	Diethylamine.	Trimethylamine.

Their relationship to the hydrocarbons is shown by their method of preparation from the halogen derivatives. When a halogen derivative is treated with ammonia, the halogen atom is replaced by a NH_2 group: this new compound will again react with the halogen derivative and the process can be continued until all the hydrogen atoms of ammonia are substituted by alkyl groups:—

$$CH_3 . Cl + NH_3 = HCl + CH_3 . NH_2.$$
$$CH_3 . Cl + CH_3 . NH_2 = HCl + CH_3 . NH . CH_3.$$
$$CH_3 . Cl + CH_3 . NH . CH_3 = HCl + CH_3 . N(CH_3) . CH_3.$$

The three compounds so obtained are termed primary, secondary, and tertiary amines according as one, two, or three of the hydrogen atoms in ammonia are replaced by the alkyl groups. Like ammonia they are strong bases and form salts with acids. The basic character increases as the number of alkyl groups in the molecule increases. They are very stable compounds and are not easily broken up into their constituents. They are readily distinguished by the action of nitrous acid:—

The primary amine is converted into the corresponding alcohol with evolution of nitrogen:—

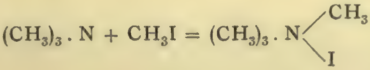
$$CH_3 . NH_2 + O : N . OH = CH_3 . OH + N_2 + H_2O.$$

The secondary amine is converted into a nitroso derivative:—

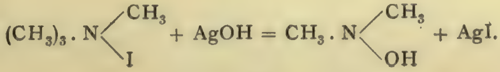
$$CH_3 . NH . CH_3 + O : N . OH = CH_3 . N(N : O) . CH_3 + H_2O.$$

The tertiary amine is not acted upon.

The tertiary amines can combine with another molecule of halogen derivative and in this compound the halogen atom can be replaced by an OH group:—

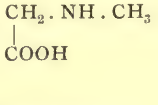


$$(CH_3)_3 . N + CH_3I = (CH_3)_3 . N\left\langle\begin{matrix}CH_3\\ I\end{matrix}\right.$$



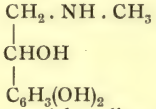
$$(CH_3)_3 . N\left\langle\begin{matrix}CH_3\\ I\end{matrix}\right. + AgOH = CH_3 . N\left\langle\begin{matrix}CH_3\\ OH\end{matrix}\right. + AgI.$$

Primary amines, except the amino acids (p. 102), are not or only seldom represented amongst physiological compounds. Sarcosine and adrenaline are representatives of secondary amines. Choline, muscarine, and betaine are representatives of the group of tertiary amines:—



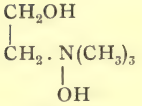
$$\begin{array}{l} CH_2 . NH . CH_3 \\ | \\ COOH \end{array}$$

Sarcosine.



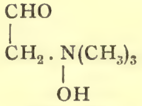
$$\begin{array}{l} CH_2 . NH . CH_3 \\ | \\ CHOH \\ | \\ C_6H_3(OH)_2 \end{array}$$

Adrenaline.



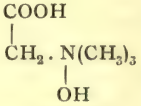
$$\begin{array}{l} CH_2OH \\ | \\ CH_2 . N(CH_3)_3 \\ \quad\quad | \\ \quad\quad OH \end{array}$$

Choline.



$$\begin{array}{l} CHO \\ | \\ CH_2 . N(CH_3)_3 \\ \quad\quad | \\ \quad\quad OH \end{array}$$

Muscarine.

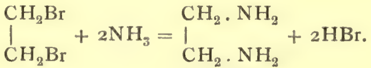


$$\begin{array}{l} COOH \\ | \\ CH_2 . N(CH_3)_3 \\ \quad\quad | \\ \quad\quad OH \end{array}$$

Betaine.

Choline or oxyethyl-trimethyl ammonium hydroxide is a trimethylamine derivative of ethyl alcohol: it occurs in lecithin, a constituent of the lipoid or fatty substance of the animal organism, which is present in egg-yolk, nervous tissue and also in other organs.

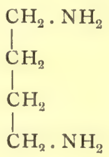
In these compounds only one nitrogen atom is present in the molecule; but corresponding to the compounds containing two or more carbon atoms and several hydroxyl groups attached to different carbon atoms, diamines, etc., occur, e.g.:—



$$\begin{array}{l} CH_2Br \\ | \\ CH_2Br \end{array} + 2NH_3 = \begin{array}{l} CH_2 . NH_2 \\ | \\ CH_2 . NH_2 \end{array} + 2HBr.$$

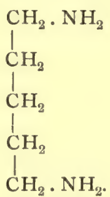
Ethylene Diamine.

The two compounds, putrescine and cadaverine, are physiological representatives of the class of diamines. They are formed from the corresponding amino acids, ornithine and lysine, by putrefaction.



$$\begin{array}{l} CH_2 . NH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CH_2 . NH_2 \end{array}$$

Putrescine.



$$\begin{array}{l} CH_2 . NH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CH_2 . NH_2. \end{array}$$

Cadaverine.

Reactions of Monomethylamine.

1. To some monomethylamine hydrochloride solution add some caustic soda and warm. Monomethylamine is evolved and is recognized by its odour like that of ammonia, alkaline reaction to litmus, and its inflammability (unlike ammonia) :—

$$CH_3 . NH_2 . HCl + NaOH = CH_3 . NH_2 + NaCl + H_2O.$$

2. Acidify some of the solution of monomethylamine hydrochloride with hydrochloric acid and add some sodium nitrite solution. Nitrogen is evolved :—

$$CH_3 . NH_2 + ON . OH = CH_3OH + N_2 + H_2O.$$

Reactions of Trimethylamine.

1. On adding caustic soda to a little trimethylamine hydrochloride solution the characteristic fishy and ammoniacal odour of trimethylamine is noticeable. The gas is also inflammable and has an alkaline reaction. N.B.

2. Add some hydrochloric acid to a little trimethylamine hydrochloride solution and then some sodium nitrite. There is no action.

3. On adding phosphotungstic acid solution to an acid solution of a trimethylamine salt a white precipitate is formed. This reaction is typical of most amines. It is owing to the presence of amine groupings in proteins that phosphotungstic acid gives a precipitate with them also. Similarly potassium mercuric iodide and potassium bismuth iodide give precipitates with amines and proteins; also with alkaloids. Hence these are sometimes known as alkaloidal reagents.

THE AMINO ACIDS.

The amino acids are derivatives of the fatty acids, or the dibasic acids, where one or more of the hydrogen atoms in the chain have been replaced by the NH_2 group. They are therefore both amines and acids in their chemical nature. Where only one of each of the groups is present in the molecule the substance is neutral, the basic group neutralizing the acid group, but when either two amino groups or two acid groups with only one of the other groups are present the substance is basic or acid in character.

These compounds can at the same time contain hydroxyl (OH) groups or thio (SH) groups in their molecule and further aromatic and other radicals can be substituted for hydrogen atoms. The main characteristic is the presence of an amine group and a carboxyl group.

They form an excessively important group of compounds, since they are constituents of the proteins, where they are combined together in various proportions. (See page 145). The following have been obtained from proteins :—

A. Monoaminomonocarboxylic Acids.

Glycine, or glycocoll, or amino-acetic acid :—

$$CH_2(NH_2) . COOH.$$

Alanine, or α-aminopropionic acid :—

$$CH_3 . CH(NH_2) . COOH.$$

Serine, or β-hydroxy-α-aminopropionic acid :—

$$CH_2OH . CH(NH_2) . COOH.$$

Cysteine, or β-thio-α-aminopropionic acid :—

$$CH_2SH . CH(NH_2) . COOH.$$

Cystine, or dicysteine, or di-(β-thio-α-aminopropionic acid) :—

$$HOOC . CH(NH_2) . CH_2 . S—S . CH_2 . CH(NH_2) . COOH.$$

Phenylalanine, or β-phenyl-α-aminopropionic acid :—

$$C_6H_5 . CH_2 . CH(NH_2) . COOH.$$

Tyrosine, or β-paraoxyphenyl-α-aminopropionic acid (see p. 129) :—

$$HO . C_6H_4 . CH_2 . CH(NH_2) . COOH.$$

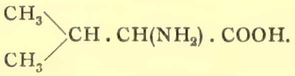
Histidine, or β-imidazole-α-aminopropionic acid :—

$$C_3H_3N_2 . CH_2 . CH(NH_2) . COOH.$$

Tryptophane, or β-indole-α-aminopropionic acid (see p. 141) :—

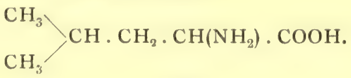
$$C_8H_6N . CH_2 . CH(NH_2) . COOH.$$

Valine, or α-aminoisovalerianic acid :—



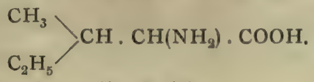
$$\begin{matrix} CH_3 \searrow \\ \quad \\ CH_3 \nearrow \end{matrix} CH . CH(NH_2) . COOH.$$

Leucine, or α-aminoisocaproic acid :—



$$\begin{matrix} CH_3 \searrow \\ \quad \\ CH_3 \nearrow \end{matrix} CH . CH_2 . CH(NH_2) . COOH.$$

Isoleucine, or β-methyl-β-ethyl-α-aminopropionic acid :—



B. Monoaminodicarboxylic Acids.

Aspartic acid, or aminosuccinic acid :—

$$HOOC.CH_2.CH(NH_2).COOH.$$

Glutamic acid, or α-aminoglutaric acid :—

$$HOOC.CH_2.CH_2.CH(NH_2).COOH.$$

C. Diaminomonocarboxylic Acids.

Ornithine, or α-, δ-diaminovalerianic acid :—

$$CH_2(NH_2).CH_2.CH_2.CH(NH_2).COOH.$$

Lysine, or α-, ε-diaminocaproic acid :—

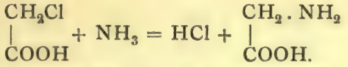
$$CH_2(NH_2).CH_2.CH_2.CH_2.CH(NH_2).COOH.$$

In addition to these compounds the substances, proline and oxyproline are found as constituents of the protein molecule (p. 140).

Preparation of Amino Acids.

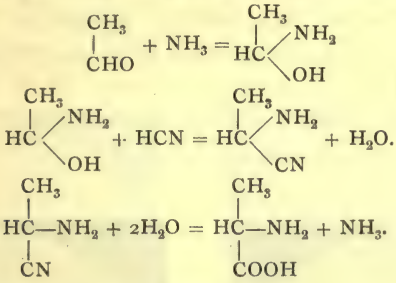
The amino acids are prepared by two methods :—

1. By the action of ammonia upon the corresponding halogen derivative :—



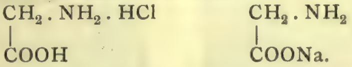
$$\begin{array}{l}CH_2Cl\\ |\\ COOH\end{array} + NH_3 = HCl + \begin{array}{l}CH_2.NH_2\\ |\\ COOH.\end{array}$$

2. By the addition of hydrogen cyanide and ammonia to aldehydes and the subsequent hydrolysis of the aminocyanohydrin :—



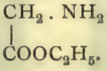
Properties of the Amino Acids.

The properties of the amino acids are very similar. They can form salts both with acids and with bases :—



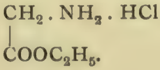
$$\begin{array}{l}CH_2.NH_2.HCl\\ |\\ COOH\end{array} \qquad \begin{array}{l}CH_2.NH_2\\ |\\ COONa.\end{array}$$

The principal derivatives are the esters, e.g. :—



$$\begin{array}{l}CH_2.NH_2\\ |\\ COOC_2H_5.\end{array}$$

These esters are mostly oils having an alkaline reaction and can be distilled in vacuo. The complex mixture of amino acids which result on the hydrolysis of proteins can be separated by means of the esters by fractional distillation. They form salts with acids :—



$$\begin{array}{l}CH_2.NH_2.HCl\\ |\\ COOC_2H_5.\end{array}$$

Glycine and the other amino acids, when obtained in a pure state, are white crystalline substances having characteristic forms :—

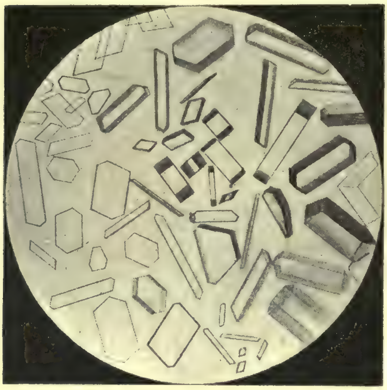


FIG. 20.—Glycine.

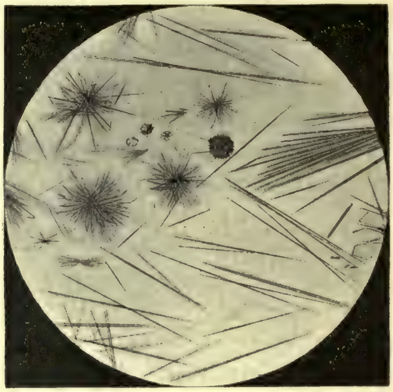


FIG. 21.—Leucine.

(From Funke's "Atlas of Physiological Chemistry".)

Glycine, when combined with benzoic acid, constitutes *hippuric acid*, $C_6H_5 . CO . NH . CH_2 . COOH$, a compound found in the urine of man, but chiefly in the urine of herbivora. It is a product formed synthetically by the kidney from its two constituents, the benzoic acid (*see later*) being obtained from aromatic substances in the food.

Glycine also occurs in the animal body in combination with cholic or cholalic acid, which is present in bile. Both hippuric acid and glycocholic acid are white crystalline substances; their crystalline form is represented in figs. 22 and 23.

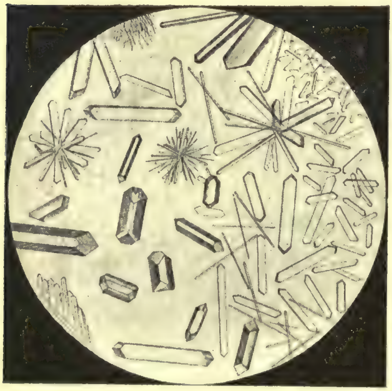


FIG. 22.—Hippuric Acid.

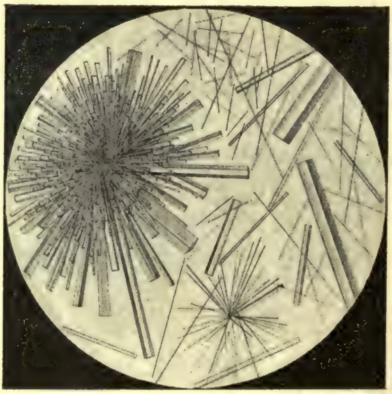
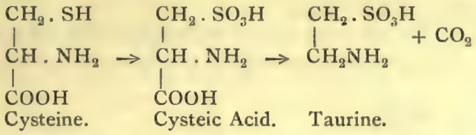


FIG. 23.—Glycocholic Acid.

(After Funke.)

Glycine is present in the greatest amount in gelatin; alanine, valine, leucine, and isoleucine are present in most, if not all, proteins; also serine. Phenylalanine, tyrosine, tryptophane are the representatives of the aromatic substances in proteins. Aspartic and glutamic acid occur most frequently in plants. Lysine, arginine and histidine are basic substances having an alkaline reaction. Each contains six carbon atoms. They are frequently termed the hexone bases.

The presence of sulphur in the molecule of cystine affords a means of readily distinguishing it from other amino acids, more especially as the sulphur is held in very loose combination and is evolved as hydrogen sulphide when cystine is boiled with alkalies. The formation of hydrogen sulphide when proteins are boiled with alkali is due to the presence of cystine. Cystine forms characteristic crystals –hexagonal plates (fig. 24). It can be recognized in this way when present in urinary deposits—especially in cases of cystinuria. In the animal body cystine (or cysteine) is probably converted into taurine:—



$$\begin{array}{l}CH_2 . SH\\ |\\ CH . NH_2\\ |\\ COOH\end{array} \rightarrow \begin{array}{l}CH_2 . SO_3H\\ |\\ CH . NH_2\\ |\\ COOH\end{array} \rightarrow \begin{array}{l}CH_2 . SO_3H\\ |\\ CH_2NH_2\end{array} + CO_2$$

Cysteine. Cysteic Acid. Taurine.

Taurine is present in the bile in combination with cholalic acid as taurocholic acid. Taurine is most readily prepared from dog's bile from which it crystallizes in a characteristic form (fig. 25).

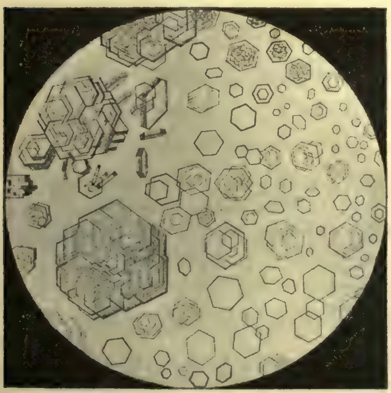


FIG. 24.—Cystine.

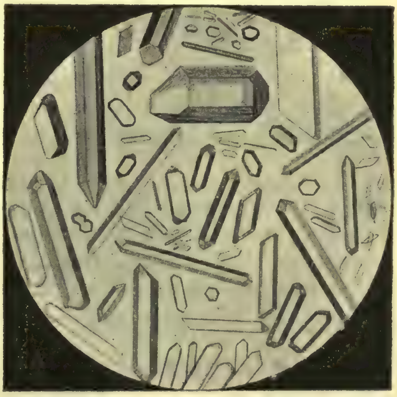


FIG. 25.—Taurine.

(After Funke.)

Reactions of Glycine.

The reactions of glycine are typical of the reactions of all the amino acids.

1. Treat a little glycine dissolved in water with some fuming nitric acid (nitrous acid) or with some sodium nitrite solution and 1 to 2 drops of dilute acid. Nitrogen is evolved showing the presence of an amino or $-NH_2$ group:—

$$CH_2(NH_2)COOH + OHNO = CH_2(OH)COOH + N_2 + H_2O.$$

P.109.

2. Boil a little glycine dissolved in water with caustic soda. Ammonia is not evolved. Glycine is not an amide (compare acetamide), but an amine in its chemical nature.

3. Dissolve a few crystals of glycine in water and add a drop or two of dilute copper sulphate solution. A blue colour is at once produced, different in shade to that of the copper sulphate and much more intense.

Amino acids are often isolated as their copper salts, by which they are characterized. They are prepared by boiling the solution of amino acid with excess of copper oxide or copper carbonate, filtering and evaporating until the solution crystallizes.

Boil a little glycine with excess of copper carbonate, filter, and evaporate to dryness. The blue copper salt of glycine crystallizes out.

Hippuric Acid.

Preparation from Urine. To 25 c.c. of herbivorous urine add 12 gm. of ammonium sulphate and 1 c.c. of concentrated hydrochloric acid. Separation of hippuric acid in crystals commences in about 5 minutes and is complete in about 10 minutes. Filter off the crystals, and dry them between sheets of filter paper. They are generally more or less pigmented.

Properties.

1. Recrystallize the hippuric acid obtained above from hot water. On cooling it separates out in the form of needles. Examine with the microscope and compare with fig. 22.

2. Heat a little of the hippuric acid in a test tube. It melts, and on further heating the mass becomes red due to the decomposition of the glycine. A sublimate of benzoic acid and the smell of bitter almonds is produced. Dissolve the sublimate in dilute sodium carbonate solution and then add dilute hydrochloric acid. The benzoic acid separates out. Examine microscopically and compare with the hippuric acid crystals.

3. Hippuric acid is hydrolyzed into its constituents by boiling with acids or alkalies:—

$$C_6H_5.CO\text{—}NH.CH_2.COOH + H_2O = C_6H_5.COOH + NH_2.CH_2.COOH.$$

Boil some hippuric acid for a few minutes with strong hydrochloric acid, cool, and filter off the crystals of benzoic acid. To the filtrate add a slight excess of ammonia, boil until the solution is neutral and the excess of ammonia is removed. On now adding a few drops of copper sulphate solution, the deep blue colour characteristic of the copper salt of glycine is formed.

Cystine.

Preparation. Folin ("J. Biol. Chem.," 1910, **8**, 9) has shown that cystine can be very readily obtained by the hydrolysis of wool.

omit.

Wool has been hydrolyzed into its constituent amino acids by boiling it with concentrated hydrochloric acid for about five hours in the proportion of 50 gm. wool to 100 c.c. acid.

To the given solution add solid sodium acetate until the reaction of the solution is no longer acid to congo red. A dark precipitate containing the cystine is thrown down. Filter this off and wash it with cold water. Then dissolve it in 5 per cent. hydrochloric acid and boil the solution with animal charcoal till it is colourless. Filter, and to the hot solution add hot sodium acetate solution. The cystine separates in the typical hexagonal plates.

Properties.

1. Examine the crystals of cystine under the microscope and compare them with fig. 24.

2. Dissolve some of the cystine in caustic soda and add a drop of lead acetate solution. Boil. Lead sulphide is formed as shown by the brown to black colour of the precipitate.

Estimation of Amino Acids.

As amino acids are neutral in reaction, they cannot be titrated by means of standard alkali like ordinary acids. They combine, however, with formaldehyde and yield an acid which can be titrated in this way; thus:—

$$\begin{array}{l} CH_2 . NH_2 \\ | \\ COOH \end{array} + OHCH = H_2O + \begin{array}{l} CH_2 . N = CH_2 \\ | \\ COOH. \end{array}$$

The process of estimation is carried out as follows:—

To 10 c.c. of commercial formalin diluted with two volumes of water add 6 to 8 drops of phenolphthalein solution and then run in $\frac{N}{10}$ caustic soda solution until the colour is pink. Now add this neutralized formalin solution to 20 or 25 c.c of amino acid solution measured out with a pipette into a small flask. The pink colour disappears. Run in $\frac{N}{10}$ caustic soda solution until a distinct pink colour again appears. Note the number of c.c. of alkali added.

If 12 c.c. were added then 25 c.c. of amino acid solution contain 12 c.c. of $\frac{N}{10}$ amino acid; hence the amount in 100 c.c. If the amino acid be known, its weight can be calculated. If a mixture of amino acids be present, the amount is best expressed in terms of nitrogen.

AMIDES.

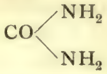
The amides are derived from ammonia by the replacement of one hydrogen atom by an acid radicle, e.g. :—

$H.CO.NH_2$

Formamide.

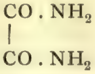
$CH_3.CO.NH_2$

Acetamide.



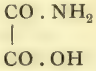
$$\mathrm{CO}\begin{smallmatrix}\diagup \mathrm{NH_2}\\ \diagdown \mathrm{NH_2}\end{smallmatrix}$$

Carbamide or urea.



$$\begin{array}{l} CO.NH_2 \\ | \\ CO.NH_2 \end{array}$$

Oxamide.



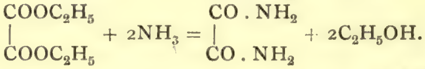
$$\begin{array}{l} CO.NH_2 \\ | \\ CO.OH \end{array}$$

Oxamic acid.

They are prepared from the corresponding acid chloride or ester by the action of ammonia thus :—

$$CH_3.CO.Cl + NH_3 = CH_3.CO.NH_2 + HCl$$

$$COCl_2 + 2NH_3 = CO(NH_2)_2 + 2HCl$$



$$\begin{array}{l} COOC_2H_5 \\ | \\ COOC_2H_5 \end{array} + 2NH_3 = \begin{array}{l} CO.NH_2 \\ | \\ CO.NH_2 \end{array} + 2C_2H_5OH.$$

The amides are thus derivatives of acids, the OH group being replaced by the amino group NH_2. The substitution of a hydrogen atom in ammonia by the acid radicle decreases its basic character; the amides are consequently weak bases and form salts only with strong acids. They are very unstable compounds being readily decomposed into their constituent acid and ammonia by boiling with acid or alkali.

Both the primary amine and the amide are characterized by the presence of the NH_2 group.

Carbamide or urea is the diamide of carbonic acid $\mathrm{CO}\begin{smallmatrix}\diagup \mathrm{OH}\\ \diagdown \mathrm{OH}\end{smallmatrix}$; the monamide $\mathrm{CO}\begin{smallmatrix}\diagup \mathrm{OH}\\ \diagdown \mathrm{NH_2}\end{smallmatrix}$ is termed carbamic acid.

Urea is the chief nitrogenous constituent of the urine; it is the end-product of mammalian metabolism being formed in the liver from ammonia and carbon dioxide. It is present in the urine to the extent of about 2 per cent. (30 gm. per day). Its crystalline form is represented in fig. 26.

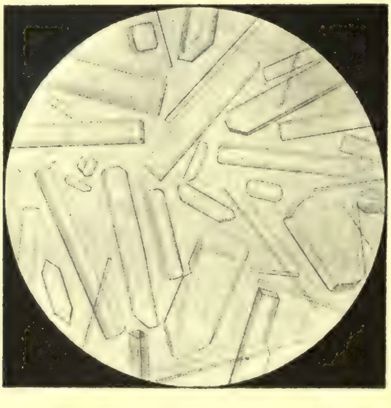


FIG. 26.—Urea. (After Funke.)

Acetamide.

Preparation. Mix together 5 c.c. of ethyl acetate and 5 c.c. of strong ammonia (sp. gr. ·880) in a small flask and allow to stand in a warm place. The two layers gradually form a single homogeneous solution.

On fractionally distilling the liquid, the ammonia is expelled, alcohol and water then pass over; subsequently the acetamide will distil over, and will solidify. Boiling point 222° C.

Reactions.

1. To some acetamide solution add a little sodium nitrite and a few drops of dilute acid. An effervescence of nitrogen occurs:—

$$CH_3.CO.NH_2 + ON.OH = CH_3.COOH + H_2O + N_2.$$

This reaction is typical of all compounds containing the NH_2 group (cf. primary amine).

2. Boil about 50 c.c. of acetamide solution with an excess of caustic soda. Ammonia is given off as recognized by the smell and the blueing of moistened red litmus paper. Continue boiling until ammonia is no longer evolved. Test the solution for acetic acid by exactly neutralizing with hydrochloric acid and adding ferric chloride. Red coloration and precipitation on heating.

N·B

3. Acetamide is hydrolyzed by heating with acid in the same way; ammonia can be detected by adding excess of magnesium oxide (to neutralize the acid) and boiling:—

$$CH_3.CONH_2 + H_2O = CH_3.COOH + NH_3.$$

Oxamide.

Preparation. To about 1 c.c. of ethyl oxalate add excess of ammonia. Oxamide is precipitated. Filter it off and wash it free from ammonia. Then dry it between sheets of filter paper.

Properties. Boil the precipitate of oxamide for some time with caustic soda. Ammonia is evolved and the oxamide, which is insoluble, gradually passes into solution as sodium oxalate.

Carbamide or Urea.

Omit

Synthesis. Make a solution of approximately equal parts of ammonium sulphate and potassium cyanate, boil for a short time and then evaporate to dryness on the water bath. Extract the dry residue with alcohol and filter from potassium sulphate. Evaporate off the alcohol from the filtrate on the water bath; urea is left as residue:—

$$(NH_4)_2SO_4 + 2KCNO = 2NH_4CNO + K_2SO_4$$
$$NH_4CNO = CO(NH_2)_2.$$

Reactions.

1. Note that urea is easily soluble in water and alcohol, but insoluble in ether.

2. Boil some urea solution with excess of caustic soda. Ammonia is evolved (amide):—

$$CO(NH_2)_2 + 2H_2O = CO_2 + H_2O + 2NH_3.$$

$NaNO_2$

3. To some urea solution add fuming nitric acid (nitrous acid) or a little sodium nitrite and a drop or two of dilute acid. Effervescence of nitrogen and carbon dioxide occurs:—

$$CO(NH_2)_2 + 2HNO_2 = CO_2 + 3H_2O + 2N_2.$$

(only white ppte
add effervescence

4. To some urea solution add a little alkaline sodium hypobromite solution; effervescence of nitrogen and carbon dioxide again occurs:—

$$CO(NH_2)_2 + 3NaOBr = CO_2 + N_2 + 3NaBr + 2H_2O$$
$$CO_2 + 2NaOH = Na_2CO_3 + H_2O.$$

In alk sol CO_2 absorbed
and no gas is
[illegible]

(only yellowish
which [illegible]
[illegible]

Note.—An effervescence also occurs when sodium hypobromite is added to ammonium chloride.

5. Dissolve a few crystals of urea in a drop of water on a watch glass. Then let a drop of concentrated nitric acid come in contact with it. Crystals of urea nitrate are formed. These consist of rhombic six-sided platelets, often imbricated (like tiles) when examined under the microscope (fig. 27):—

$$CO(NH_2)_2 + HNO_3 = CO(NH_2)_2 . HNO_3.$$

This reaction is made use of in the preparation of urea from urine.

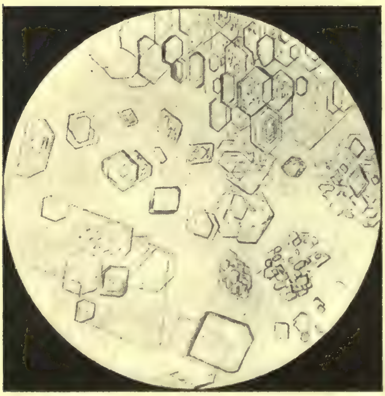


FIG. 27.—Urea nitrate.

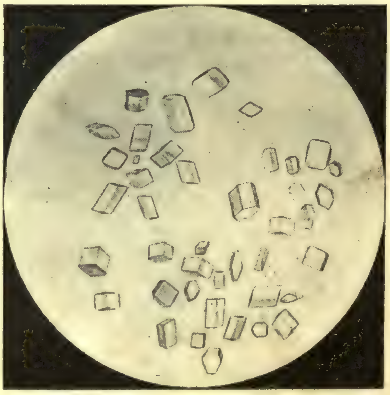
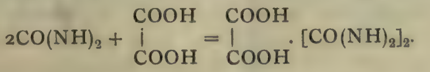


FIG. 28.—Urea oxalate.

(After Funke.)

6. Perform the same experiment using saturated oxalic acid solution instead of nitric acid. Crystals of urea oxalate are formed. When microscopically examined they consist of short rhombic prisms (fig. 28).



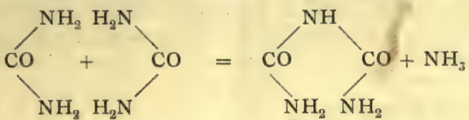
$$2CO(NH)_2 + \begin{array}{c}COOH\\|\\COOH\end{array} = \begin{array}{c}COOH\\|\\COOH\end{array} . [CO(NH)_2]_2.$$

$Hg(NO_3)_2$

7. To a little dilute urea solution add some dilute mercuric nitrate solution (1 per cent.). A white precipitate of $CO(NH_2)_2 . Hg(NO_3)_2 + HgO$ is formed.

8. Heat gently a little urea in a dry test tube; it melts; continue the heating until it turns solid, white, and opaque. Note that during the heating ammonia is given off, and that a ring of sublimed cyanuric acid may be formed on the cooler parts of the test tube. The white residue consists chiefly of biuret, but contains also cyanuric acid. Extract the mass with water, pour off the aqueous extract and test it with caustic soda and 1 or 2 drops of dilute copper sulphate (1 per cent.). A pink colour is formed (the "biuret" reaction).

Dissolve the residue in 1 or 2 drops of ammonia and add a little barium chloride; on shaking, barium cyanurate is precipitated as a white mass.

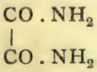


Biuret.

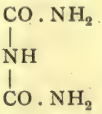
$$3CO(NH_2)_2 = C_3H_3N_3O_3 + 3NH_3.$$

Cyanuric acid.

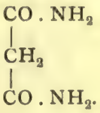
The biuret reaction is given by all compounds which contain two —CO . NH groups, either joined together directly by the carbon atoms, as in oxamide, or by a N atom, as in biuret, or by a C atom, as in malonamide :— N.B.



$$\begin{array}{c}CO . NH_2\\|\\CO . NH_2\end{array}$$



$$\begin{array}{l}CO . NH_2\\|\\NH\\|\\CO . NH_2\end{array}$$



$$\begin{array}{l}CO . NH_2\\|\\CH_2\\|\\CO . NH_2.\end{array}$$

It is also given by substances where several of these groupings occur, as in certain polypeptides and in the proteins, for which it is a distinctive reaction.

Preparation of Urea from Urine.

omit, 15
[illegible] c.

1. Evaporate 100 c.c. of urine to a syrupy consistence (to about $\frac{1}{6}$); thoroughly cool by immersing in cold water, and, keeping the mixture cold, add excess of concentrated nitric acid. Stir vigorously during the addition of the acid. Filter off the crystals of urea nitrate; dry them by pressing between successive sheets of filter paper. When the crystals of urea nitrate are dry, weigh them and calculate the yield of urea (123 gm. urea nitrate contains 60 gm. urea) from the urine. Then prepare the urea from them by mixing with excess of barium carbonate and adding a little alcohol to form a paste. The urea nitrate is decomposed, giving barium nitrate, carbon dioxide and urea. Dry the paste on the water bath, and extract the mass with alcohol. Filter. Evaporate the filtrate on the water bath nearly to dryness; urea crystallizes out in long silky needles. Prove that the substance is urea by the action of nitrous acid, or by the biuret reaction.

2. Evaporate some urine (about 25 c.c.) completely to dryness on the water bath. Extract the residue with about 10 c.c. of boiling acetone, and pour off the acetone into a clean evaporating basin. Urea crystallizes out on cooling in long silky needles. Filter and determine the yield of urea by this method, as compared with the above.

Note.—Urea oxalate may be prepared from human urine by saturating it with oxalic acid (1 gm. to 10 c.c.); the yield is about 1 per cent. (Roaf.)

Estimation of Urea.

Urea is hydrolyzed by water, acids, or alkalies into carbonic acid and ammonia; it is decomposed by nitrous acid or alkaline hypobromite into carbon dioxide and nitrogen. These reactions are made use of in order to determine the amount of urea in a given solution. The most accurate method is the hydrolysis by hydrochloric acid followed by the distillation of the ammonium chloride with excess of caustic soda and estimation of the ammonia by collecting in excess of standard acid. The most simple and rapid method is the decomposition by alkaline hypobromite: the carbon dioxide is absorbed by the alkali and the evolved nitrogen is collected and measured. This method is the one suggested by Hüfner and most frequently employed in medical practice. The estimation is generally carried out in the apparatus designed by Dupré and known as Dupré's ureometer (fig. 29).

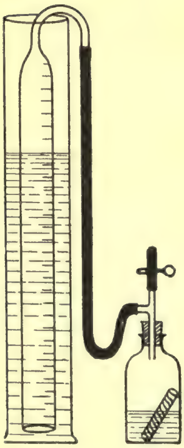


FIG. 29.

The estimation is carried out as follows:—

Place 25 c.c. of freshly-prepared hypobromite solution (100 gm. caustic soda are dissolved in 250 c.c. water and to the cold liquid 25 c.c. bromine are added) in the bottle of about 120 c.c. capacity. Measure out 5 c.c. urea solution with a pipette into a small tube and place it in the bottle, taking great care not to upset the solution into the hypobromite. Tightly cork the bottle with the india-rubber stopper and place it in cold water to cool. Through the india-rubber stopper a glass T-piece passes. One end of this is connected by rubber tubing to a graduated burette which is placed in a jar of water. The rubber tubing is of such a length that the burette can be lifted out of the water without stretching. The other end of the T-piece is closed by a piece of rubber tubing and a small clip. Fill the burette with water by opening the screw clip; raise or lower it until the water stands at the uppermost graduation and at the same level outside and inside. Then close the clip and test for leakage in the system by raising and lowering the burette in the water for at least a minute and then seeing that the level of the water returns to the top graduation when the water inside and outside the tube are again made to stand at the same level. When the system has been tested to see that it is air-tight the analysis can be commenced. Take the reading of the top graduation. Tilt the bottle so as to upset the urea solution in the little tube into the hypobromite solution and thoroughly wash it out with the latter. Nitrogen is rapidly evolved, and displaces the water in the burette. The bottle must now be brought to its original temperature by placing it for a few minutes in a fresh supply of cold water. As soon as it is cool, raise the burette till the level of the water is the same inside and outside and read the level. The difference in the readings gives the volume of nitrogen evolved. By making the levels inside and outside the burette the same, this volume is measured at the atmospheric pressure. Take the temperature of the water and ascertain the barometric pressure and the tension of aqueous vapour at that temperature. Convert the volume of nitrogen to the volume at 0° and 760 mm. Thus the corrected volume is:—

Correct temperatu
only.

$$\frac{V \times 273 \times (B-T)}{(273+t) \times 760}$$

where V = volume of gas evolved, B = barometric pressure, T = tension of aqueous vapour (p. 251), and t = temperature of water.

The amount of urea corresponding to this volume is given by the equation:—

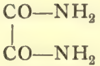
$$\underbrace{CO(NH_2)_2}_{\substack{60\ \text{gm.} \\ 1\ \text{gm.}}} + 3NaBrO + 2NaOH = 3NaBr + \underbrace{N_2}_{\substack{22{\cdot}4\ \text{litres}\ (=28\ \text{gm.}) \\ 373\ \text{c.c.}}} + Na_2CO_3 + 3H_2O.$$

Actually, however, only 354 c.c. nitrogen are evolved by 1 gm. of urea so that the method is not quite accurate; this should be allowed for.

UREIDES.

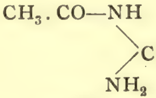
Just as ammonia forms amides with acids so also does urea form ureides, thus:—

$CH_3CO—NH_2$
Acetamide.

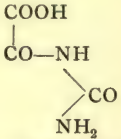


$$\begin{array}{l}CO—NH_2\\ |\\ CO—NH_2\end{array}$$

Oxamide.

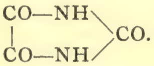


Acetylurea.



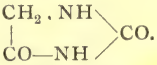
Oxaluric acid.

With the dibasic acids the combination may occur with both carboxyl groups as in oxalylurea, or parabanic acid:—

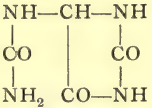


This compound is the simplest representative of the group, and is an oxidation product of uric acid.

Not only does urea form ureides with dicarboxylic acids, but also with hydroxy acids, or oxy-acids, thus with glycollic acid it forms hydantoin, and with glyoxylic acid it forms allantoin, a physiological compound found first in the allantoic fluid and recently shown to be formed by the liver from uric acid:—

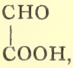


Hydantoin.



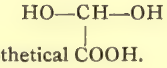
Allantoin.

Allantoin is a combination of two molecules of urea with glyoxylic acid,



$$\begin{array}{l}CHO\\ |\\ COOH\end{array}$$

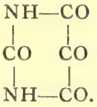
, being derived from the hypothetical



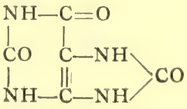
$$\begin{array}{c}HO—CH—OH\\ |\\ COOH\end{array}$$

.

A very important oxidation product of uric acid is alloxan,



Uric acid, the principal ureide biologically, is a combination of an hydroxy acid containing three carbon atoms with two molecules of urea; it has the formula:—



PURINES.

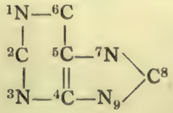
Uric acid belongs to the group of compounds known as the purines to which the physiological compounds xanthine, hypoxanthine, guanine, adenine also belong. Caffeine or theine, and theobromine, the active constituents of tea, coffee, and cocoa, are also members of this group. Guanine and adenine are constituents of the nucleic acid contained in cell nuclei. They are converted by the enzymes guanase and adenase into xanthine and hypoxanthine respectively. Xanthine and hypoxanthine are further oxidized by the body into uric acid.

Uric acid occurs in the urine of man to the extent of 0·5 to 1 gramme per day; under pathological conditions its amount is greater. It is the chief nitrogenous excretory product of birds and reptiles. The urine of these animals is our chief source of this compound.

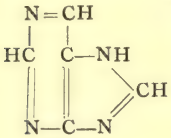
All these compounds have been synthesized by Emil Fischer and their chemical relationship to one another thus established. The result of these investigations has shown that they are all derived from the compound, purine, which stands in the same kind of relationship to them as a hydrocarbon does to an alcohol, an amine, etc. Thus

Purine	$C_5H_4N_4$
Hypoxanthine	$C_5H_4N_4O$
Xanthine	$C_5H_4N_4O_2$
Uric acid	$C_5H_4N_4O_3$
Adenine	$C_5H_3N_4 . NH_2$
Guanine	$C_5H_3N_4 . NH_2 . O$
Theobromine	$C_5H_2N_4(CH_3)_2O_2$
Caffeine	$C_5HN_4(CH_3)_3O_2$.

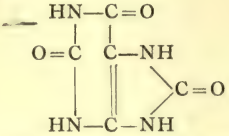
The compounds have, however, a ring structure (compare benzene and its derivatives), i.e. a complex of atoms which undergo no change in position and which can be numbered in order thus :—



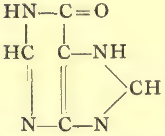
The formulæ for the various compounds are :—



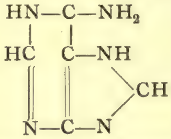
Purine.



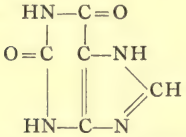
Uric acid, or 2, 6, 8, trioxypurine.



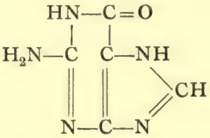
Hypoxanthine or 6, monoxypurine.



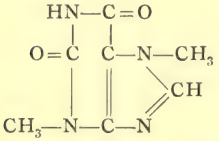
Adenine, or 6, aminopurine.



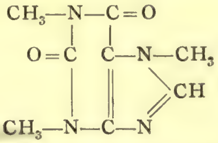
Xanthine, or 2, 6, dioxypurine.



Guanine, or 2, amino- 6, oxypurine.



Theobromine, or 3, 7, dimethyl-, 2, 6, dioxypurine.



Caffeine, or 1, 3, 7-trimethyl-, 2, 6, dioxypurine.

Preparation of Uric Acid from Snake's Urine, or Bird's Guano.

Dissolve some snake's excrement or bird's guano in a small quantity of dilute caustic soda solution, filter from insoluble material (sand, etc.), add hydrochloric acid in excess to the filtrate and cool. A precipitate of uric acid is formed. Examine the crystals under the microscope and compare them with the various forms shown in fig. 30. Filter off the precipitate and wash it free from hydrochloric acid with water. Dry the uric acid between sheets of filter paper. Carry out the following reactions with the product so obtained.

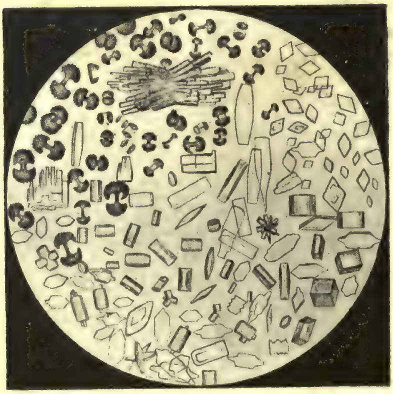


Fig. 30.—Uric acid. (After Funke.)

Properties of Uric Acid.

1. Uric acid is almost insoluble in cold water, more readily in hot water.

2. It dissolves in dilute caustic soda (on warming); on acidifying with hydrochloric acid, if the solution be sufficiently concentrated, it is precipitated; this precipitate dissolves on warming and reappears on cooling. Sodium urate crystallizes from concentrated solutions in the form shown in fig. 31.

3. A solution of sodium urate on saturating with ammonium chloride gives a precipitate of ammonium urate. Perform this experiment. Compare the appearance of the precipitate with fig. 32. This reaction is utilized in the estimation of uric acid in urine (p. 236).

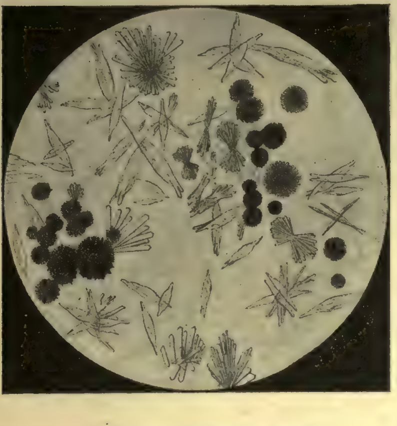


FIG. 31.—Sodium urate.

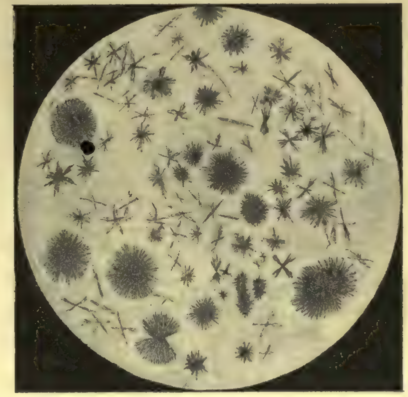


FIG. 32.—Ammonium urate.

(After Funke.)

4. To a very dilute solution of uric acid in caustic soda, add ammonia and then magnesia mixture and ammoniacal silver nitrate. A gelatinous white precipitate of silver magnesium urate is formed.

This reaction is sometimes used to separate uric acid from urine together with the purine bases also present in the urine.

Whyco
Sod

5. A white precipitate of cuprous urate is formed on adding copper sulphate and sodium bisulphite to an alkaline solution of uric acid.

6. Uric acid reduces Fehling's solution on boiling for some time. Heat some uric acid with Fehling's solution, and note that on prolonged boiling cuprous oxide is precipitated.

Note.—On this account, urine on prolonged boiling reduces Fehling's solution, and the reduction may be attributed to glucose in small amounts. (See under Pathological Urines.)

7. Schiff's test. Dissolve some uric acid in a little sodium carbonate solution and pour some of the solution upon a filter paper moistened with silver nitrate. The silver nitrate is reduced leaving a black stain of metallic silver.

8. Murexide test. To a small quantity of uric acid on a crucible lid or porcelain basin add a few drops of strong nitric acid. Heat gently over a small flame until the nitric acid has been evaporated and the yellow residue begins to turn red. Allow it to cool, and then with a glass rod bring a drop of ammonia or caustic soda to the yellow residue—a purple-red colour is formed. With caustic soda the colour is more blue.

Preparation of Uric Acid from Human Urine.

1. 500 c.c. of urine have been treated with 50 c.c. of concentrated hydrochloric acid and allowed to stand for 24 hours in a cool place. Pigmented crystals of uric acid will be found adhering to the sides of the vessel. Examine them under the microscope; they consist of irregular crystals, much pigmented, and generally arranged in sheaves (pp. 116, 241).

2. Saturate 100 c.c. of urine with crystals of ammonium chloride (27 gm. necessary) and add 1 to 2 drops of strong ammonia. After fifteen minutes filter off the gelatinous precipitate of ammonium urate. Test for the presence of uric acid in a small portion of this precipitate by the murexide test, i.e. by scraping off the precipitate, placing it in an evaporating basin, adding 3 to 4 drops of concentrated nitric acid, and evaporating to dryness. A yellow-red stain results, which becomes purple on adding ammonia.

The uric acid is obtained by dissolving the remainder of the precipitate in the smallest possible quantity of hot water, filtering if necessary, and then adding a drop of concentrated hydrochloric acid. Uric acid separates on cooling if too much water has not been used in dissolving the ammonium urate.

Estimation of Uric Acid.

Uric acid is readily oxidized by potassium permanganate and converted into allantoin. Use is made of this reaction for its estimation. $\frac{N}{20}$ $KMnO_4$ is commonly used in this process.

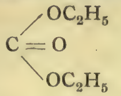
Place the standard permanganate solution in the burette with glass tap (rubber cannot be used as it is attacked by permanganate). Read the level by holding a lighted match behind it. Measure out with a pipette 100 c.c. of the solution of sodium urate or the same volume of a known weighed quantity of uric acid (about 0·5 gm. dissolved in 1000 c.c. of water containing a small quantity of soda), add 20 c.c. of concentrated sulphuric acid, mix well and run in the permanganate at once without cooling.

During the titration the liquid in the flask must be agitated continuously; at first every drop of permanganate is decolorized almost at once before it diffuses through the liquid. As soon as a pink flush is seen throughout the solution the end point is reached. This colour soon disappears, but another drop of permanganate will diffuse before it disappears. This can be continued almost indefinitely so that the first pink flush must be carefully looked for.

1 c.c. $KMnO_4$ = 0·00375 gm. uric acid.
∴ x c.c. = $x \times 0{\cdot}00375$ gm. uric acid in 100 c.c. solution.

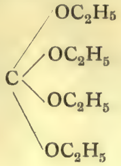
GUANIDINE AND GUANIDINO ACIDS.

Amides are formed by the action of ammonia upon esters (p. 108); urea results by its action on ethyl carbonate or carbonic ester:—



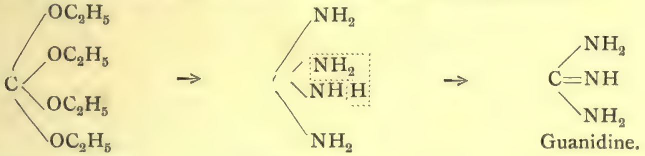
$$C{=}O(OC_2H_5)_2$$

Besides this ester of carbonic acid, the compound,



$$C(OC_2H_5)_4$$

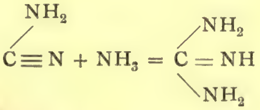
which is termed orthocarbonic ester, has been prepared. Guanidine is obtained by the action of ammonia upon this compound:—



$$C(OC_2H_5)_4 \rightarrow C(NH_2)_2(NH_2)(NHH) \rightarrow C(NH_2)_2{=}NH$$

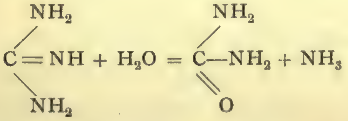
Orthocarbonic ester. Hypothetical. Guanidine.

Guanidine is however generally prepared by the action of ammonia upon cyanamide:—



$$NH_2C{\equiv}N + NH_3 = C{=}NH(NH_2)_2$$

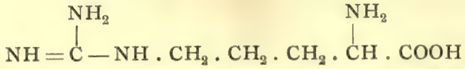
Guanidine is thus urea in which the O atom has been replaced by the $=NH$ group. It is a strongly basic substance and forms salts readily with acids, e.g. guanidine carbonate is formed when guanidine is exposed to the air. It is hydrolyzed by alkalies into urea and ammonia:—



$$C{=}NH(NH_2)_2 + H_2O = C(NH_2)_2{=}O + NH_3$$

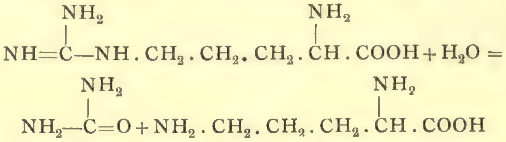
Guanidine is known chiefly by synthesis: it has been found in nature in certain plants. Two of its derivatives are extremely important biological compounds.

Arginine,



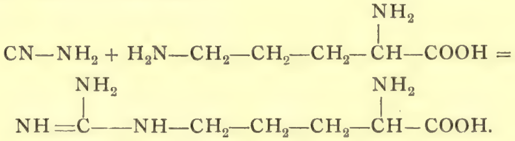
$NH{=}\overset{NH_2}{\overset{|}{C}}{-}NH.CH_2.CH_2.CH_2.\overset{NH_2}{\overset{|}{C}H}.COOH$

Arginine, or δ-guanidine-*a*-aminovalerianic acid is a constituent of the protein molecule; in some proteins—the protamines—it forms over 80 per cent. of the molecule (p. 145). Like guanidine it is a basic substance and is hydrolyzed by alkalies into urea and ornithine:—



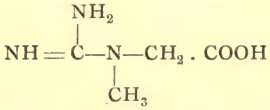
$$NH{=}\overset{NH_2}{\overset{|}{C}}{-}NH.CH_2.CH_2.CH_2.\overset{NH_2}{\overset{|}{C}H}.COOH+H_2O=$$
$$NH_2{-}\overset{NH_2}{\overset{|}{C}}{=}O+NH_2.CH_2.CH_2.CH_2.\overset{NH_2}{\overset{|}{C}H}.COOH$$

This hydrolysis of arginine occurs in the liver by the action of the enzyme arginase. A portion of the urea in the urine is probably derived in this way. Its constitution was proved by its synthesis from cyanamide and ornithine:—



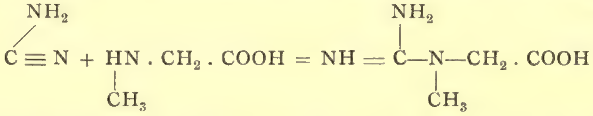
$$CN{-}NH_2+H_2N{-}CH_2{-}CH_2{-}CH_2{-}\overset{NH_2}{\overset{|}{C}H}{-}COOH=$$
$$NH{=}\overset{NH_2}{\overset{|}{C}}{-}NH{-}CH_2{-}CH_2{-}CH_2{-}\overset{NH_2}{\overset{|}{C}H}{-}COOH.$$

Creatine,



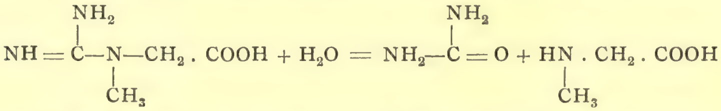
$NH{=}\overset{NH_2}{\overset{|}{C}}{-}\underset{CH_3}{\underset{|}{N}}{-}CH_2.COOH$

Creatine, or methyl guanidine acetic acid, occurs to a large extent in muscle and under certain conditions appears in the urine. It has been synthesized from cyanamide and sarcosine, or methyl-glycine:—



$$\overset{NH_2\diagup}{C}{\equiv}N+\underset{CH_3}{\underset{|}{HN}}.CH_2.COOH=NH{=}\overset{NH_2}{\overset{|}{C}}{-}\underset{CH_3}{\underset{|}{N}}{-}CH_2.COOH$$

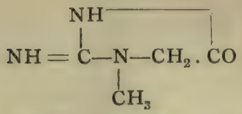
It is hydrolyzed by alkalies into urea and sarcosine and may, therefore, like arginine, contribute to the quantity of urea in urine:—



$$NH{=}\overset{NH_2}{\overset{|}{C}}{-}\underset{CH_3}{\underset{|}{N}}{-}CH_2.COOH+H_2O=NH_2{-}\overset{NH_2}{\overset{|}{C}}{=}O+\underset{CH_3}{\underset{|}{HN}}.CH_2.COOH$$

Creatine can easily be prepared from meat by extracting it with hot water, removing the proteins from solution by precipitation with lead acetate, and evaporating the solution to a small volume after removal of excess of lead by hydrogen sulphide and filtration from lead sulphide. Creatine crystallizes out in rhombic prisms on standing (fig. 33):—

Creatinine,



$$NH = \overset{\displaystyle NH\text{———————}\,\,|}{\overset{|}{C}} - \underset{\underset{\displaystyle CH_3}{|}}{N} - CH_2 . \overset{|}{CO}$$

Creatinine is the anhydride of creatine and is obtained from creatine by boiling with acids. The reverse reaction takes place when creatinine is treated with alkalies. Creatinine is a constituent of the urine, its average daily amount being about 1 gramme.

From the chemical point of view its origin in the urine would be from the creatine of muscle, but the physiological experiments do not bear out this relationship; the daily amount of creatinine in urine is very constant and its amount is not increased by adding creatine to the diet: when taken in large doses creatine itself appears in the urine.

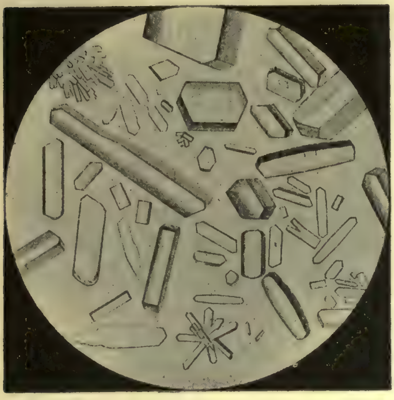


Fig. 33.—Creatine.

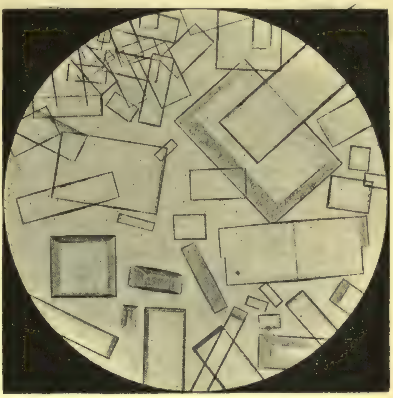


Fig. 34.—Creatinine

(After Funke.)

Creatinine forms a distinctive double compound with zinc chloride; it has also two distinctive reactions by which its presence (and also that of creatine) can be detected.

Detection of Creatinine in Urine.

1. Weyl's Reaction.

To a small quantity of urine add a few drops of dilute freshly prepared sodium nitro-prusside solution, and make alkaline with caustic soda. The solution becomes red in colour. Acidify strongly with acetic acid; this decolorizes the solution. Heat. The solution acquires a green tint and a blue deposit forms on standing.

2. Jaffé's Reaction.

Add a little saturated picric acid solution to some urine in a test tube, and render alkaline with caustic soda. The solution becomes deep orange in colour. This change of colour may be hastened by warming.

Detection of Creatine and Creatinine in Meat Extract.

A fresh extract of meat contains creatine, but an extract, such as Liebig's, contains creatinine which is probably formed by the action of the acid in the extract during evaporation.

1. To 10 c.c. of meat extract solution add 10 c.c. of caustic soda solution and 15 c.c. of saturated picric acid solution. Allow to stand for 5 minutes: then dilute to 200 c.c.; orange colour if creatinine be present. Keep the solution for comparison with 2 and 3.

2. Heat the same quantity of meat extract solution on the water bath for 15 minutes with 10 c.c. caustic soda solution. Then add 15 c.c. picric acid solution, allow to stand 5 minutes and dilute to 200 c.c. No orange colour, as creatinine has been converted into creatine.

P. 121.

3. Heat a third quantity of 10 c.c. of meat extract solution with 5 c.c. of dilute hydrochloric acid for about half an hour on the water bath. Then neutralize, add caustic soda and picric acid as before, and dilute to 200 c.c.; The orange colour is deeper than in 1 due to the conversion of creatine into creatinine.

p. 121.

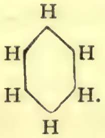
AROMATIC COMPOUNDS.

The aromatic compounds, benzene and its derivatives, are mostly obtained from the aromatic oils and resins, which are contained in the flowers, leaves and other parts of plants and often ooze out when the bark, etc., is broken. Benzene itself is obtained from coal tar, which is a complex mixture of aromatic compounds.

Though only a few aromatic compounds are actually found in the animal organism, yet they are essential to life. The aniline dyes, the essential oils (scents), india-rubber, and numerous pharmacological compounds are benzene derivatives. The alkaloids, indigo, etc., are more complex compounds.

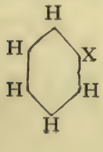
The aromatic compounds differ from the previous compounds of the fatty or aliphatic series by their greater carbon content, and by containing a nucleus made up of six carbon atoms. This nucleus is extremely stable; oxidation, reduction, etc., can be carried on without its being destroyed. The production of nitro-compounds, by the direct action of nitric acid, is particularly characteristic of benzene derivatives, whereas the aliphatic compounds are generally oxidized and decomposed.

Benzene, C_6H_6, is the simplest compound, and is generally represented as a regular hexagon; at each angle is situated a carbon atom and a hydrogen atom; by substituting different groups and side chains for the hydrogen atoms, all other aromatic compounds may be obtained:—

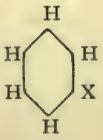


This representation of benzene as a simple hexagon shows that the hydrogen atoms in the molecule are of equal value and it explains most simply the existence of the various isomers amongst its substitution derivatives, thus:—

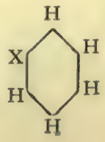
Mono-substitution derivatives only exist in one form



is the same as



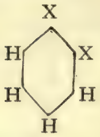
and



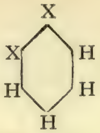
etc.

Disubstitution derivatives exist in three forms :—

1. Ortho



or



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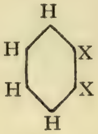
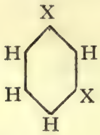


etc.

2. Meta



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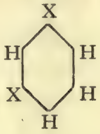

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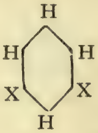
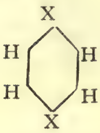


etc.

3. Para



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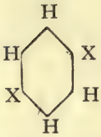

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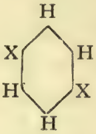
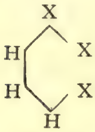
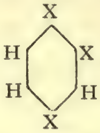


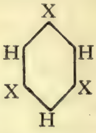
Tri-substitution derivatives also exist in three forms :—



Vicinal.



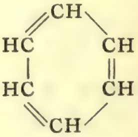
Asymmetric.



Symmetric.

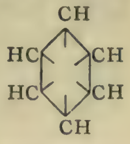
Tetra-substitution derivatives exist in three forms, penta in one, and hexa in one. In the above forms the substituting radicles X are identical ; if not identical, the number of possible isomers is still greater.

It has been previously stated that the element carbon is tetra-valent, but in this representation of the benzene nucleus no account is taken of this property. Kekulé, who originally proposed this method of representing benzene and its derivatives, suggested that the carbon atoms were alternately linked together by double and single bonds, thus :—

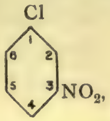


on this representation two isomeric mono-substitution derivatives would be possible. To overcome this difficulty Kekulé assumed that there was a continual alternation between the double and single bonds. The double bonds again are not double bonds like those in unsaturated aliphatic compounds.

Other chemists have assumed a different figure such as a prism for the benzene nucleus; this does not explain the substitution derivatives as well as the centric formula proposed by Armstrong, in which the bonds not accounted for simply point to the centre of the figure, and come into play only under certain conditions, e.g. amongst the hydroaromatic compounds.



For simplification, the benzene nucleus is generally written without the carbon and hydrogen atoms, their existence at the angles being recognized. The substitution products are then indicated by introducing only the particular radicle or radicles. Further the carbon atoms are generally numbered in order. The compound,



is thus 1, chloro-3, nitro-benzene or metachloro-nitro-benzene.

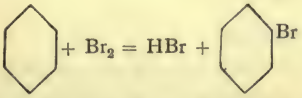
BENZENE AND ITS MONOSUBSTITUTION DERIVATIVES.

It was previously stated that benzene was obtained from coal tar. Coal tar consists of a complex mixture of aromatic compounds which are acid, basic, and neutral in character. By shaking out with acid the basic substances are removed; then by shaking out with alkali the acid substances are separated, amongst them phenol, cresol. The neutral substances remain. After drying they are fractionally distilled; the various fractions contain benzene, toluene, xylene, naphthalene, anthracene, etc.

Benzene is contained in the first fraction; when pure it is an oily, colourless liquid boiling at 80° C.

Chlorobenzene C_6H_5Cl. **Bromobenzene** C_6H_5Br.

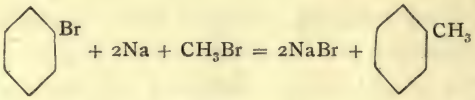
Like the aliphatic hydrocarbons benzene is acted upon by the halogens, chlorine and bromine, and converted into substitution derivatives. These are formed by the direct action of the halogen on benzene, the reaction proceeding most easily in the presence of a halogen carrier such as iodine (iodine chloride is formed which chlorinates more vigorously):—



Unlike the corresponding aliphatic halogen compounds, these compounds are more stable; the halogen atom is not replaceable by OH or other groups.

Toluene or Methyl-Benzene $C_6H_5 . CH_3$. **Ethyl Benzene** $C_6H_5 . C_2H_5$.

Toluene is contained in the second fraction of the products of distillation of the neutral substances of coal tar. It can be obtained from bromobenzene and methyl bromide by the action of sodium :—



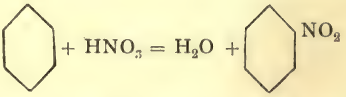
Ethyl benzene and the higher homologues are obtained by the same reaction from the alkyl bromides and from bromobenzene.

Toluene is a colourless liquid boiling at 110° C. It is known commercially as toluole; benzene is known as benzole, and xylene (p. 128) as xylole. The terminal e is added to show that the compounds do not contain a hydroxyl (OH) group, *ol* being the suffix to designate these compounds.

These homologues of benzene contain both a benzene nucleus and an aliphatic radicle or side chain. They can therefore behave both as aromatic compounds and as aliphatic compounds. As aromatic compounds they will form nitro and sulphuric acid derivatives with nitric and sulphuric acids (see nitrobenzene and benzene sulphonic acid). As aliphatic compounds they will react with halogens to give substitution products, e.g. $C_6H_5 . CH_2Cl$ which give hydroxy compounds $C_6H_5 . CH_2OH$ when treated with caustic potash. On oxidation they give aldehydes $C_6H_5 . CHO$ and acids $C_6H_5 . COOH$. Whatever be the length of the side chain and whatever groups it may contain its reactions are those of the compounds of the aliphatic series.

Nitrobenzene. $C_6H_5 . NO_2$.

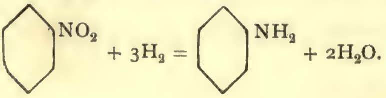
By the action of nitric acid on benzene, benzene is converted into nitrobenzene :—



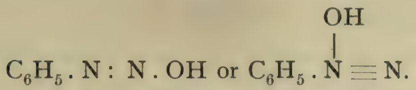
This reaction, as previously mentioned, is one of the principal reactions which benzene and its derivatives undergo and in which they differ from aliphatic compounds.

Aminobenzene or Aniline. $C_6H_5 . NH_2$.

By the action of reducing agents on nitrobenzene, the NO_2 group is converted into the NH_2 group, and aniline is formed :—

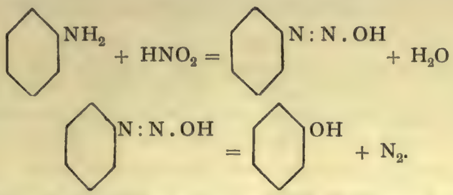


Diazobenzene. $C_6H_5 . N : N . OH$ or



$C_6H_5 . \overset{\overset{OH}{|}}{N} \equiv N.$

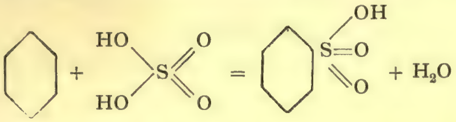
Aniline is a primary amine. Primary amines of the aliphatic series are converted by the action of nitrous acid into the corresponding alcohol. By the action of nitrous acid on aniline, the substance diazobenzene is first formed; this compound is unstable and passes into phenol:—



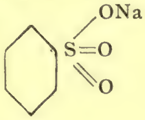
Diazobenzene behaves like a base (NH_4OH) and forms salts with acids (NH_4Cl).

Benzene Sulphonic Acid. $C_6H_5 . SO_3H$.

By the action of sulphuric acid on benzene or its derivatives, sulphonation occurs (compare nitration with nitric acid).

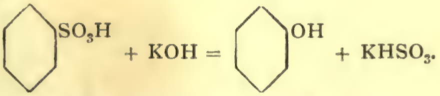


The resulting compound is an acid and forms salts with alkalies:—

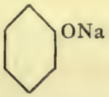


Phenol. $C_6H_5 . OH$.

As above stated phenol is obtained by the decomposition of diazobenzene. It is also obtained by fusing benzene sulphonic acid with caustic potash:—



Phenol is contained amongst the acid substances of coal tar. It is an example of an aromatic alcohol. Unlike the aliphatic alcohols the aromatic alcohols are acid in character and form salts with caustic alkalies which are stable:—



On this account phenol is commercially known as carbolic acid. Phenol is present in the urine of mammals as its sulphuric acid ester (p. 44); it originates from tyrosine by oxidation (p. 129).

Benzoic Acid. $C_6H_5.COOH$.

Benzoic acid is formed when the mono-substituted homologues of benzene, i.e. those containing a side chain, are oxidized. This oxidation can be effected in the laboratory by the action of potassium permanganate. The same oxidation occurs in the animal body. The presence of benzoic acid in the urine in combination with glycine as hippuric acid is due to the oxidation of aromatic substances in the food. Thus, toluene and ethyl benzene undergo the following stages in their oxidation :—

$$\underset{\text{Toluene.}}{C_6H_5.CH_3} \rightarrow \underset{\text{Benzylalcohol.}}{C_6H_5.CH_2OH} \rightarrow \underset{\text{Benzaldehyde.}}{C_6H_5.CHO} \rightarrow \underset{\text{Benzoic acid.}}{C_6H_5.COOH.}$$

$$\underset{\text{Ethylbenzene.}}{C_6H_5.CH_2.CH_3} \rightarrow \underset{\text{Phenylacetic acid.}}{C_6H_5.CH_2.COOH} \rightarrow \underset{\text{Benzoic acid.}}{C_6H_5.COOH.}$$

In the more complex compounds one carbon atom after the other is removed as carbon dioxide ; any side chain, however long, is ultimately oxidized to benzoic acid. Benzoic acid is only further oxidized by the strongest oxidizing agents.

DISUBSTITUTION DERIVATIVES.

These exist in three forms, the ortho, meta, and para.

Dimethylbenzenes or Xylenes.

The three xylenes occur in coal tar from which they are obtained by fractional distillation.

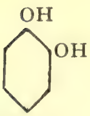
Dibromobenzenes.

By the bromination of benzene, a mixture of the ortho and para compounds is obtained : meta-bromobenzene is obtained by indirect methods.

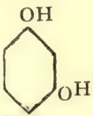
Dinitrobenzenes.

By the further nitration of benzene, metadinitrobenzene is formed.

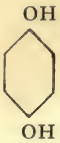
Dihydroxybenzenes.



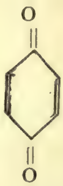
Ortho.
Pyrocatechin or catechol.



Meta.
Resorcinol.

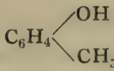


Para.
Hydroquinone or quinol.

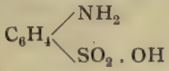


Quinone.

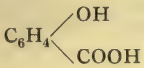
The pyrocatechin and hydroquinone nuclei are contained in the physiological substances adrenaline and homogentisic acid. Resorcinol is used in the preparation of eosin. Hydroquinone is easily oxidized to quinone and finds extensive use in photography; pyrocatechin behaves very like hydroquinone. They are all white crystalline substances and have characteristic reactions.

Cresols. 
$C_6H_4\left\langle\begin{matrix}OH\\CH_3\end{matrix}\right.$

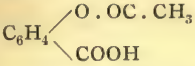
The para compound is of interest physiologically. Its ester with sulphuric acid occurs in the urine and it originates from tyrosine by oxidation. It behaves very like phenol.

Sulphanilic Acid. 
$C_6H_4\left\langle\begin{matrix}NH_2\\SO_2\,.\,OH\end{matrix}\right.$

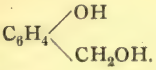
Para-sulphanilic acid is obtained by heating aniline with sulphuric acid. It is an aromatic amino-acid and is used extensively in the dye industry. It is diazotized by treating with nitrous acid; the diazo compound is then condensed with various phenols and anilines. The azo-dyes are thus made.

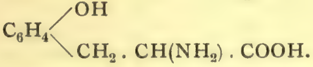
Salicylic Acid. 
$C_6H_4\left\langle\begin{matrix}OH\\COOH\end{matrix}\right.$

Only the ortho compound is of importance: it occurs in nature as free acid and as methyl ester (oil of winter green); it is used largely medicinally. Aspirin is acetyl salicylic acid,

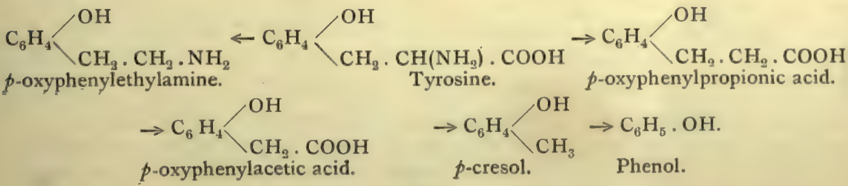

$$C_6H_4\left\langle\begin{matrix}O\,.\,OC\,.\,CH_3\\COOH\end{matrix}\right.$$

The glucoside salicin contains the corresponding alcohol, salicyl alcohol or saligenin,


$$C_6H_4\left\langle\begin{matrix}OH\\CH_2OH.\end{matrix}\right.$$

Tyrosine. 
$C_6H_4\left\langle\begin{matrix}OH\\CH_2\,.\,CH(NH_2)\,.\,COOH.\end{matrix}\right.$

Tyrosine is parahydroxyphenyl-*a*-aminopropionic acid. It is another example of an aliphatic amino acid containing an aromatic radicle in its molecule. It is a constituent of certain proteins, and it is owing to its presence that these proteins give the xanthoproteic and Millon's reactions. (See proteins.) Proteins which do not contain tyrosine cannot maintain life. It is the mother substance of phenol and cresol in the urine. Further, one of the active principles of ergot, *p*-hydroxyphenylethylamine, has its origin in tyrosine. The vital oxidation of tyrosine has been thoroughly investigated by Baumann, who showed that it gave rise to cresol and phenol by the following stages:—


$$C_6H_4\left\langle\begin{matrix}OH\\CH_2\,.\,CH_2\,.\,NH_2\end{matrix}\right. \leftarrow C_6H_4\left\langle\begin{matrix}OH\\CH_2\,.\,CH(NH_2)\,.\,COOH\end{matrix}\right. \rightarrow C_6H_4\left\langle\begin{matrix}OH\\CH_2\,.\,CH_2\,.\,COOH\end{matrix}\right.$$

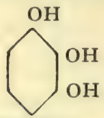
p-oxyphenylethylamine. Tyrosine. *p*-oxyphenylpropionic acid.

$$\rightarrow C_6H_4\left\langle\begin{matrix}OH\\CH_2\,.\,COOH\end{matrix}\right. \rightarrow C_6H_4\left\langle\begin{matrix}OH\\CH_3\end{matrix}\right. \rightarrow C_6H_5\,.\,OH.$$

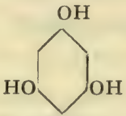
p-oxyphenylacetic acid. *p*-cresol. Phenol.

Tri- and Tetra-substitution Derivatives.

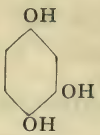
The following compounds are found in nature and are of importance biologically and practically :—



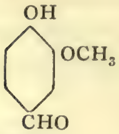
Pyrogallol.



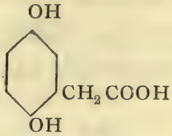
Phloroglucinol.



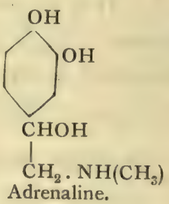
Oxyhydroquinone.



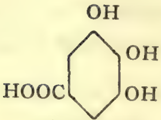
Vanillin.



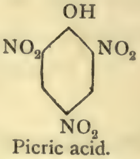
Homogentisic acid.



Adrenaline.



Gallic acid.



Picric acid.

Preparation of Nitrobenzene.

To a mixture of 2 c.c. of concentrated sulphuric acid and 1 c.c. of concentrated nitric acid add a drop of benzene and warm. Nitrobenzene is formed, and is recognized by its smell of bitter almonds, which remains after removing the excess of acid by alkali.

Preparation of Aniline.

To a drop of nitrobenzene add 2 portions of stannous chloride (each sufficient to cover the tip of a penknife) and 3 to 4 c.c. of caustic soda. Warm the mixture for some time. The nitrobenzene smell disappears giving place to that of aniline :—

$$C_6H_5NO_2 + 3H_2 = C_6H_5 . NH_2 + 2H_2O.$$

Shake the alkaline solution with ether, pour off the layer of ether into a basin and allow it to evaporate on the water bath. Treat the residue with bleaching powder solution; a purple-violet colour appears which becomes dirty red.

Properties of Aniline.

1. Aniline is soluble with difficulty in water, but dissolves on adding acid, forming salts; on adding caustic soda the aniline again separates in oily drops: —

$$C_6H_5 . NH_2 + HCl = C_6H_5 . NH_2HCl.$$

2. Bromine water gives with an aqueous solution of aniline a pinkish precipitate (which becomes grey-green), of tribromaniline :—

$$C_6H_5NH_2 + 3Br_2 = C_6H_2Br_3 . NH_2 + 3HBr.$$

Preparation and Properties of Diazobenzene.

To a cooled solution of aniline hydrochloride add a few drops of sodium nitrite solution. Diazobenzene chloride is formed; on warming this is decomposed, giving phenol with evolution of nitrogen :—

$$C_6H_5 . NH_2 . HCl + HNO_2 = C_6H_5 . N : N . Cl + H_2O.$$
$$C_6H_5 . N_2 . Cl + H_2O = C_6H_5OH + N_2 + HCl.$$

Properties of Phenol.

1. To a little phenol solution add a drop of ferric chloride solution. There is a blue violet coloration which disappears on adding mineral acids (HCl).

2. To some phenol solution add a little bromine water. There is first a cloudiness due to mono- or dibromophenol which is characterized by a very penetrating smell. The further addition of bromine water produces a precipitate of tribromophenol in yellowish-white needles or flakes. Tribromophenol is formed directly with dilute solutions of phenol.

3. To a little phenol solution add concentrated nitric acid and warm. A yellowish colour is produced. Cool and add ammonia: the colour is intensified and becomes orange. Picric acid is formed :—

$$C_6H_5 . OH + 3HNO_3 = C_6H_2(NO_2)_3OH + 3H_2O.$$

N.B.

4. Add Millon's reagent (mercury dissolved in concentrated nitric acid giving mercuric and mercurous nitrate and nitrite; the latter is essential) and heat. There is a deep red coloration.

N.B.

Detection of Phenol in Urine. (Roaf.)

To 5 c.c. of horse's urine add 0·5 c.c. of concentrated hydrochloric acid and boil for at least two minutes to hydrolyze the ester. Then cool thoroughly and extract the solution with ether. Separate the ethereal layer and allow the ether to evaporate. Dissolve the residue in water and test it with Millon's reagent.

Properties of Picric Acid.

Picric acid is obtained by treating phenol with concentrated nitric acid (p. 131). It is a yellow crystalline solid, soluble in water.

1. Boil a little picric acid solution with glucose and caustic soda. The colour becomes red, owing to the reduction of the picric acid to picramic acid :—

$$C_6H_2(NO_2)_3 . OH + 3H_2 = C_6H_2(NO_2)_2 . NH_2 . OH + 2H_2O.$$

✓ 2. Picric acid dyes animal fibres (wool, silk) permanently yellow, but not plant fibres (cotton). Dip pieces of wool and cotton in picric acid solution, then wash them with water; the wool retains the yellow colour, whereas the stain on the cotton washes out.

3. Picric acid precipitates basic substances and is used for precipitating alkaloids and proteins (see proteins).

Properties of Benzoic Acid.

1. Dissolve a little benzoic acid in caustic soda. Acidify the solution with hydrochloric acid; the benzoic acid is precipitated in a crystalline form, and on boiling goes into solution, reappearing again on cooling.

2. Dissolve a little benzoic acid in ether; allow the ether to evaporate in the cold; benzoic acid separates in a crystalline form.

✓ 3. Heat a little benzoic acid in a test tube; it melts and gives off white suffocating fumes with characteristic smell; it condenses on the sides of the tube as a white crystalline mass.

✓ 4. Mix intimately a little benzoic acid with soda lime and heat in a dry test tube; benzene is formed, which has a characteristic smell and burns with a smoky flame. (Cf. acetic acid, p. 35) :—

$$C_6H_5 . COOH + Ca(OH)_2 = C_6H_6 + CaCO_3 + H_2O.$$

5. On evaporating a little benzoic acid with nitric acid in a porcelain basin, nitro-benzene is formed, as recognized by its smell :—

$$C_6H_5 . COOH + HNO_3 = C_6H_5 . NO_2 + CO_2 + H_2O.$$

2.3. ✓ 6. Note that benzoic acid does not give a reaction with bromine water, nor a yellow colour reaction with nitric acid, nor the reaction with Millon's reagent. These reactions are typical of an -OH group in the benzene ring. (Cf. cresol, salicylic acid.)

Properties of Pyrocatechin and Hydroquinone.

1. Pyrocatechin gives a green coloration with ferric chloride. If a drop of ammonia be now added, or better, a trace of tartaric acid and then ammonia, the green colour changes to violet.

Hydroquinone when boiled with ferric chloride is converted into quinone, which has a peculiar characteristic smell.

2. Both pyrocatechin and hydroquinone reduce ammoniacal silver nitrate in the cold.

3. On adding caustic soda to solutions of pyrocatechin and hydroquinone they become brown, firstly at the surface, but on shaking, throughout the whole solution. This is due to absorption of oxygen.

4. They reduce Fehling's solution slowly. On boiling decolorization occurs, but only a scanty separation of cuprous oxide takes place.

5. Add Millon's reagent to some pyrocatechin and hydroquinone and heat; hydroquinone gives a yellow colour and then a yellow precipitate, which becomes red on heating.

Properties of Cresol.

As it is a phenol, cresol reacts :—

1. With ferric chloride.
2. With bromine water.
3. With nitric acid.
4. With Millon's reagent.

Properties of Sulphanilic Acid.

1. Treat a little cold saturated solution of sulphanilic acid with dilute sodium nitrite solution and dilute sulphuric acid, and warm. Nitrogen is evolved as with aniline, the intermediate diazobenzene sulphonic acid being decomposed.

2. Dilute 1 to 2 drops of the cold saturated solution of sulphanilic acid with 10 c.c. water, add a drop of alcoholic α-naphthol solution, a drop of dilute sodium nitrite solution, and a drop of dilute sulphuric acid. A yellow-red colour (dye) is produced, which becomes purple-red on adding ammonia.

Properties of Salicylic Acid.

It is soluble with difficulty in cold water, but easily soluble in hot from which it crystallizes on cooling.

When mixed with soda lime and heated, it gives phenol, as recognized by its smell.

As it is a phenol it gives the following reactions :—

1. A yellow colour when heated with nitric acid, which, when cold, becomes orange on adding ammonia.
2. A white cloudy precipitate with bromine water.
3. A red colour when heated with Millon's reagent.
4. A violet colour with ferric chloride; this is discharged by mineral acids.

Properties of Tyrosine.

1. It is with difficulty soluble in cold water; easily soluble in ammonia and hydrochloric acid. If a little be dissolved in ammonia on a glass slide and allowed to evaporate, tyrosine separates in bunches of fine needles. Observe them under the microscope and compare with fig. 35.

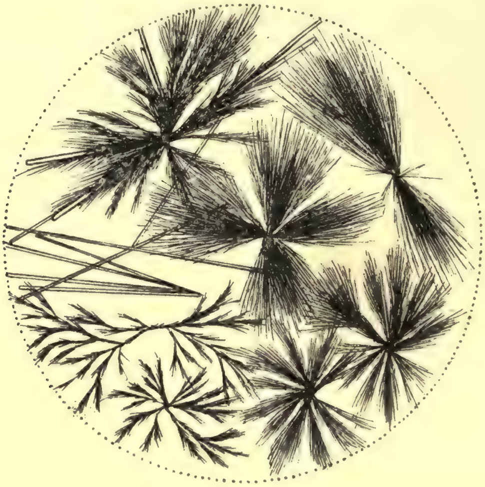


Fig. 35.—Tyrosine.

2. It gives a yellow colour on heating with nitric acid, which becomes orange on adding ammonia.

3. It gives a red colour even in extreme dilution when heated with Millon's reagent.

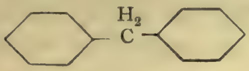
4. Put 3 drops of concentrated sulphuric acid on a little tyrosine in a dry test tube and place in the boiling water bath for half an hour. Then add 5 c.c. of water and neutralize with barium carbonate or magnesium oxide. The filtrate (from $BaSO_4$) gives a blue-violet colour with 2 to 3 drops of ferric chloride. (Piria's test.) Tyrosine is thus a phenol.

5. When boiled with copper carbonate it gives a blue copper salt like other aliphatic amino acids.

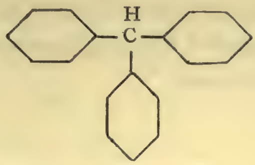
COMPLEX AROMATIC COMPOUNDS.

The preceding compounds all contain only one benzene nucleus. Numerous compounds are known which contain two or more benzene nuclei. The majority are artificial substances and have been prepared in the laboratory from the products contained in coal tar; a few are found in nature.

The benzene nuclei in these compounds may either be joined together by a carbon atom, e.g. :—

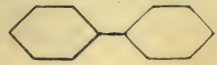


Diphenylmethane.

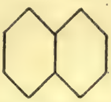


Triphenylmethane.

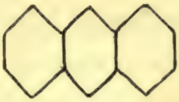
or they may be joined directly together, e.g. :—



Diphenyl.

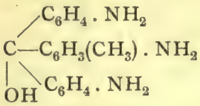


Naphthalene.



Anthracene.

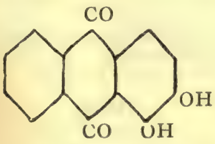
Triphenylmethane is the mother substance of the rosaniline dyes; the benzene rings are substituted by amino groups, e.g. :—



$$\begin{array}{l} \diagup C_6H_4 \, . \, NH_2 \\ C - C_6H_3(CH_3) \, . \, NH_2 \\ | \diagdown C_6H_4 \, . \, NH_2 \\ OH \end{array}$$

Rosaniline.

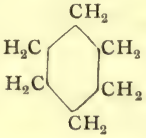
Anthracene is the mother substance of alizarin, the red colouring matter of madder, which is a dihydroxy derivative and a quinone.



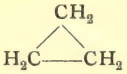
Alizarin.

HYDROAROMATIC COMPOUNDS.

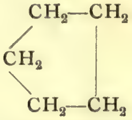
The unsaturated nature of the benzene ring is shown by the formation of hexahydrobenzene when benzene is reduced with hydriodic acid :—



This compound contains six methylene (CH_2) groups and is generally termed hexamethylene, so as to conform to the terminology of the compounds,



Trimethylene.



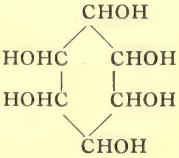
Pentamethylene.

which contain respectively three and five methylene groups joined together in the form of a ring.

A very large number of natural substances belong to the group of the hydroaromatic compounds ; they are mostly of vegetable origin (turpentine oil, camphor, scents) ; but some are present in animal organisms (inositol, cholesterol, etc.) ; our knowledge of the former has advanced considerably, but our knowledge of the latter is very slight. It appears from recent results that these compounds play a distinct rôle in the mechanism of the action of alkaloids and of toxins on the animal body.

Inositol. $C_6H_{12}O_6$.

Inositol (inosite) is found in muscle : it has the composition of a carbohydrate and was formerly termed muscle sugar ; it has the constitution of hexahydroxyhexamethylene :—

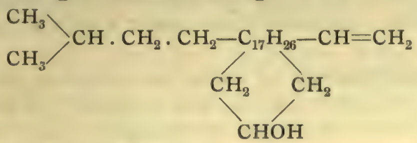


Its crystalline form is represented in fig. 36.

Cholesterol.

Cholesterol occurs in all animal tissues in conjunction with the fats and lecithin and is grouped amongst the compounds known as lipoids (soluble in media in which fats are soluble). It is present to the greatest extent in brain and nervous tissue and is the chief constituent of gall-stones.

Similar compounds are found in plants and are known as phytosterols. Cholesterol is a monatomic, or monohydric, secondary alcohol and the following formula is assigned to it :—



$$\genfrac{}{}{0pt}{}{CH_3}{CH_3}\!>\!CH\,.\,CH_2\,.\,CH_2-\underset{\underset{CHOH}{CH_2\qquad CH_2}}{C_{17}H_{26}}-CH{=}CH_2$$

It is supposed to contain four reduced benzene nuclei.

Cholesterol is readily obtained in a crystalline state; rhombic plates with a notched angle separate out when alcohol is used as the solvent. They are represented in fig. 37.

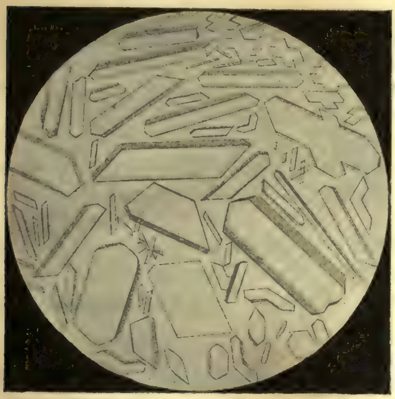


FIG. 36.—Inositol.

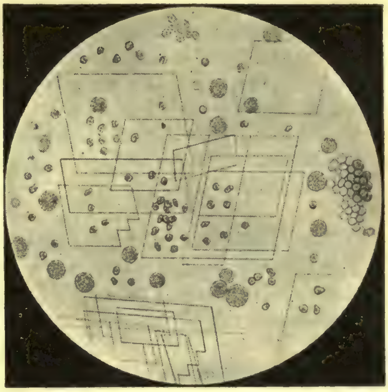


FIG. 37.—Cholesterol.

(After Funke.)

Preparation of Cholesterol.

1. **From Brain.** Mix a sheep's brain with plaster of Paris so that it forms a dry mass (or dry a sheep's brain by spreading it out in a fine layer on glass plates and then scraping off the dry material). Extract the dry mass twice with cold acetone, sufficient only being used to just cover the material. Allow the acetone to evaporate off (or distil off). Cholesterol separates in crystals. Examine them under a microscope.

2. **From Gall-stones.** Powder up a piece of gall-stone and extract the mass with a little ether. Filter and evaporate off the ether. Examine the crystals so obtained under the microscope. They consist of fine white needles.

Properties of Cholesterol.

As above seen cholesterol is soluble in acetone, chloroform and ether; from the latter solvent it crystallizes in needles. It is also soluble in alcohol, but insoluble in water.

Dissolve a little cholesterol in hot alcohol. Place a few drops on a watch glass and examine the crystals which separate out under the microscope. They consist of rhombic plates with the characteristic notched angle (fig. 37).

As an alcohol, cholesterol forms esters: the acetic and benzoic esters are characteristic and serve as a means for identifying cholesterol, thus:—

1. **Cholesteryl Acetate.** Boil a little cholesterol with acetic anhydride and fused sodium acetate for a few minutes. Then pour the mixture into water. Cholesteryl acetate is precipitated. When recrystallized from alcohol it melts at 114° C.

2. **Cholesteryl Benzoate.** Heat a little cholesterol with benzoyl chloride for some minutes and pour into alcohol. Cholesteryl benzoate is precipitated. Recrystallize it from hot alcohol. This compound melts at 145° C. to a turbid liquid, which becomes clear at 178° to 180°; on cooling it exhibits a display of colours, of which blue is the most marked.

Cholesterol may also be identified by the following colour reactions :—

1. Put some cholesterol crystals on a glass slide and cover them with a cover-slip. Then run in a drop of sulphuric acid (5 vols. concentrated sulphuric acid to 1 vol. water) under the cover-slip, and draw through with filter paper; at the edge of the cover-slip place a drop of iodine solution in potassium iodide. Owing to the action of the sulphuric acid, the crystals become red, but as the iodine solution comes into contact with them, the colour changes to violet and finally to blue.

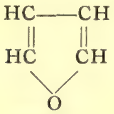
2. **Salkowski's reaction.** Dissolve a little cholesterol in a small quantity of chloroform, add an equal volume of concentrated sulphuric acid and mix the two fluids. The chloroform which rises to the surface is coloured red and the sulphuric acid is yellow and shows a green fluorescence. If the chloroform solution be poured into a dry test tube and a drop of water be added, it is decolorized, but the red colour appears again on adding concentrated sulphuric acid. It is only stable in the presence of strong sulphuric acid.

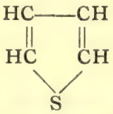
3. **Liebermann's reaction.** Dissolve a little cholesterol in 2 c.c. of chloroform in a dry test tube, add ten drops of acetic anhydride and two drops of concentrated sulphuric acid. Shake. The solution becomes red, then blue, and finally bluish-green in colour.

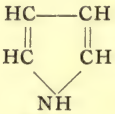
HETEROCYLIC COMPOUNDS.

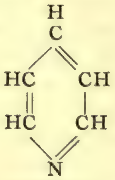
Ring compounds such as benzene and its derivatives, in which all the atoms composing the ring are the same, are known as homocyclic compounds.

Numerous compounds exist which contain rings composed of carbon and nitrogen (the purine ring, p. 115) or carbon and oxygen, or carbon and sulphur atoms, e.g.

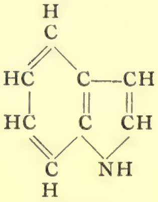

Furane.

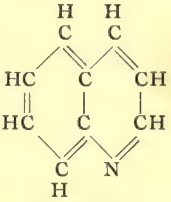

Thiophene.

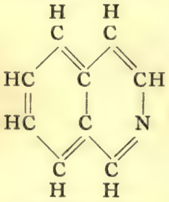

Pyrrole.


Pyridine.

These rings again may be combined with a benzene ring. Thus


Indole.

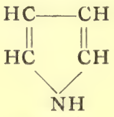

Quinoline.

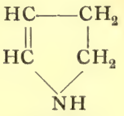

Isoquinoline.

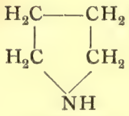
The hydrogen atoms in these ring compounds may be substituted for various groups and radicles just as those in the benzene ring.

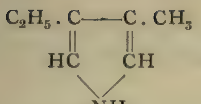
Furfural, $C_4H_3O.CHO$, the aldehyde of furane, is obtained by distilling pentoses with acids, and use is made of this reaction for estimating pentoses in physiological liquids.

The thiophene ring is not yet known to be represented amongst natural compounds. Thiophene is present in coal tar and is generally present in benzene. Proline and oxyproline, constituents of the protein molecule, are derivatives of pyrrole; so also is hæmopyrrole, which is contained in hæmoglobin and chlorophyll:—

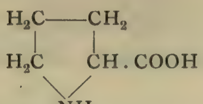

Pyrrole.


Pyrroline.

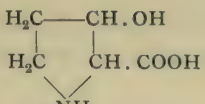

Pyrrolidine.



β-β′-methylethyl pyrrole
or
hæmopyrrole.

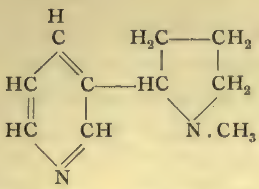


α-pyrrolidine
carboxylic acid
or
proline.



β-oxy-α-
pyrrolidine
carboxylic acid
or
β-oxyproline.

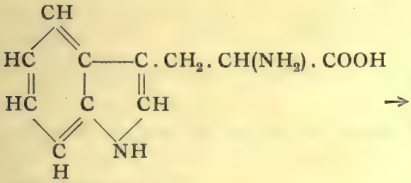
The vegetable alkaloid nicotine contains a pyrrole ring and a pyridine ring:—



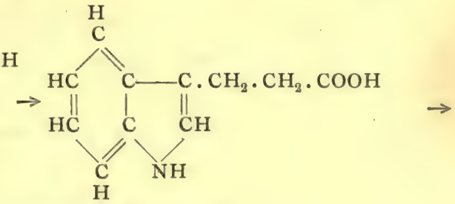
Other vegetable alkaloids contain quinoline and isoquinoline rings.

The indole ring is present in indole and scatole, indoxyl, indican, tryptophane. These five compounds form a very characteristic group amongst physiological substances.

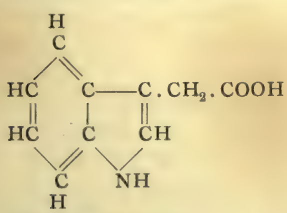
Indole and scatole are products of putrefaction of tryptophane (protein); they are derived from tryptophane by the same series of reactions as cresol and phenol are derived from tyrosine: thus



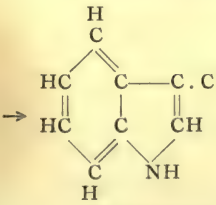
Tryptophane, or
β-indole-α-aminopropionic acid.



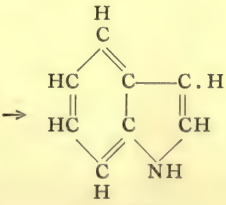
β-indolepropionic acid.



Indole acetic acid.

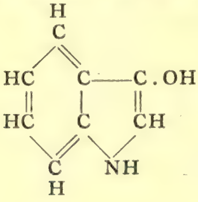


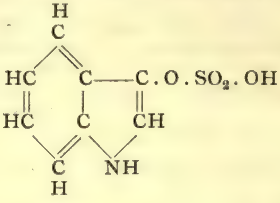
Scatole
or β-methylindole.



Indole.

Indole is oxidized in the animal body to indoxyl, which is excreted in the urine as ester with sulphuric acid (indican) or glycuronic acid.

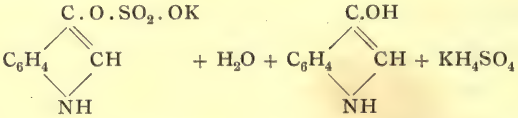

Indoxyl.


Indican.

It is detected in urine by hydrolyzing the ester with acid and oxidizing the indoxyl to indigotin or indigo-blue.

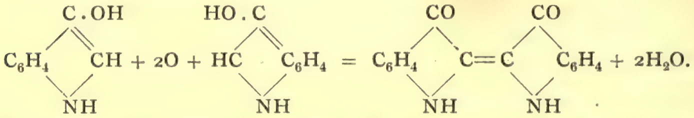
The chemical changes which occur in these reactions are :—

1. The hydrolysis of indican (potassium indoxyl-sulphate) into indoxyl and sulphuric acid :—



$$C_6H_4\overset{C.O.SO_2.OK}{\underset{NH}{\langle\rangle}}CH + H_2O + C_6H_4\overset{C.OH}{\underset{NH}{\langle\rangle}}CH + KH_4SO_4$$

2. The oxidation of indoxyl to indigotin (indigo-blue) :—



$$C_6H_4\overset{C.OH}{\underset{NH}{\langle\rangle}}CH + 2O + HC\overset{HO.C}{\underset{NH}{\langle\rangle}}C_6H_4 = C_6H_4\overset{CO}{\underset{NH}{\langle\rangle}}C{=}C\overset{CO}{\underset{NH}{\langle\rangle}}C_6H_4 + 2H_2O.$$

The plant product, indican, is a combination of indoxyl with glucose, i.e. it is a glucoside.

All these compounds give distinctive reactions by which they may be recognized in solution.

I. Indole.

In the products of distillation of putrefied meat, indole may be detected as follows :—

1. To the aqueous solution add a small quantity of nitric acid and then carefully sodium nitrite solution. The solution becomes red in colour and on standing a red precipitate of nitrosoindole (secondary amine) separates out.

2. An aqueous solution (1 : 1000) treated with sodium nitroprusside till of a yellow colour and then with a few drops of caustic soda becomes blue violet in colour. The colour changes to blue on acidifying with hydrochloric or acetic acid.

II. Indican.

Indican is detected in urine (especially herbivorous urine in which it is present in comparatively large amounts) by the following reactions :—

1. To 10 c.c. of urine add an equal volume of concentrated hydrochloric acid and 2 to 3 c.c. of chloroform. Then add, *drop by drop*, a very dilute solution of calcium hypochlorite (bleaching powder) or sodium hypochlorite and invert the solution after the addition of each drop. The chloroform becomes bluish-violet owing to the formation of indigo-blue by oxidation. Excess of hypochlorite must be avoided as the indigo-blue undergoes further oxidation to colourless compounds.

2. This further oxidation is to a large extent avoided by using ferric chloride in concentrated hydrochloric acid (1 to 2 parts in 500 concentrated hydrochloric acid) as the oxidizing agent (Obermayer's reagent).

To 10 c.c. of urine add an equal volume of Obermayer's reagent and a few c.c. of chloroform : mix thoroughly for 1 to 2 minutes by inverting the solution. The chloroform becomes blue.

3. Salkowski recommends the use of copper sulphate as the oxidizing agent as further oxidation is avoided.

To 10 c.c. of urine add an equal volume of concentrated hydrochloric acid, 1 c.c. of copper sulphate solution and a few c.c. of chloroform. Carry out as above.

Other oxidizing agents may be employed, e.g. potassium persulphate (1 drop of a 10 per cent. solution to 5 to 6 c.c. urine), potassium chlorate (1 drop of a 3 per cent. solution to 10 c.c. urine).

III. Tryptophane.

Tryptophane is readily recognized when mixed with other amino acids in solution by the following two reactions :—

1. Mix a few drops of glyoxylic acid solution with about 5 c.c. of the solution in a test tube; then run in to the bottom of the test tube concentrated sulphuric acid so as to form a layer beneath the surface of the solution. At the junction of the liquids a violet ring will appear and on gently mixing the liquids this violet colour will spread throughout the mixture.

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f. tryp
in prot.

2. Add bromine water, drop by drop, to about 5 c.c. of the solution acidified with acetic acid ; a reddish colour appears : this gradually deepens and if too much bromine water be added will disappear. When the maximum reddish colour is obtained add 2 to 3 c.c. of amyl alcohol and mix the liquids. The amyl alcohol dissolves the pigment and separates coloured red or violet.

N.B.
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tryptoph.
free sta

THE PROTEINS.

The proteins are the most important constituents of all living cells, and are therefore an essential ingredient of our food. They constitute the greater part of meat and eggs, and are present in large quantities in milk, seeds, etc.

They are composed of amino acids, which may be regarded as the units of the protein molecule. Up to the present time eighteen amino acids have been found to occur in the protein molecule (p. 102). These units are combined together in the form of acid amides, i.e. the carboxyl group of one amino acid is combined with the amino group of another amino acid, e.g. :—

Glycyl-glycine - $CH_2(NH_2) . CO—NH . CH_2 . COOH$.
Alanyl-leucine - $CH_3 . CH(NH_2) . CO—NH . CH(C_4H_9)COOH$.
Leucyl-alanine - $C_4H_9 . CH(NH_2) . CO—NH . CH(CH_3) . COOH$.

in which the units may either be the same or different, and combined in any possible order.

These combinations of amino acids have been termed by Emil Fischer the polypeptides. The above compounds are dipeptides. In the same way we may have :—

Tripeptides, e.g.

Diglycyl-glycine or glycyl-glycyl-glycine,
$CH_2(NH_2)CO—NH . CH_2 . CO—NH . CH_2 . COOH$
Glycyl-alanyl-tyrosine,
$CH_2(NH_2)CO—NH . CH(CH_3)CO—NH . CH(CH_2 . C_6H_4OH)COOH$
Alanyl-glycyl-tyrosine,
$CH_3 . CH(NH_2)CO—NH . CH_2 . CO—NH . CH(CH_2 . C_6H_4OH) . COOH$.

Tetrapeptides, e.g.

Glycyl-alanyl-glycyl-tyrosine,
$CH_2(NH_2)CO—NH . CH(CH_3)CO—NH . CH_2 . CO—NH . CH(CH_2 . C_6H_4OH)COOH$.

Pentapeptides, hexapeptides, etc.

The most complex polypeptide known is an octadecapeptide, which is composed of eighteen units made up of three leucine units and fifteen glycine units. This compound, if it had been found in nature, would undoubtedly have been regarded as a true protein.

The synthesis of these polypeptides has been effected by Emil Fischer in three ways. The simplest of these methods of combining two or more amino acids together is by the action of the acid chloride derivative of one unit upon the other unit, e.g. alanyl-leucine is formed by the action of alanyl-chloride upon leucine :—

$$\underset{\text{Alanyl chloride.}}{CH_3 . CH(NH_2) . CO . Cl} + \underset{\text{Leucine.}}{H_2N . CH(C_4H_9) . COOH} =$$

$$HCl + \underset{\text{Alanyl-leucine.}}{CH_3 . CH(NH_2) . CO—NH . CH(C_4H_9) . COOH.}$$

By the action of another amino acid chloride upon this compound a tripeptide will be formed, and the continuation of the process will lead eventually to the most complex polypeptides. The new compound (di- or tripeptide) is again an amino acid, and can be converted into its acid chloride. This acid chloride will react with another amino acid, a dipeptide, tripeptide, etc., yielding a tetra-, a penta-, a hexapeptide.

The order in which these amino acids can thus be combined together is very various. Consequently an enormous number of isomers is possible.

The evidence which we possess in support of this polypeptide constitution of the proteins is (1) the hydrolysis of these polypeptides by trypsin and other proteoclastic enzymes into their constituent units in the same way as the natural proteins are hydrolyzed, (2) the isolation of polypeptides from the natural proteins, e.g. glycyl-tyrosine.

Just as all or only a few of the units may be combined together synthetically, so also a natural protein may be composed of all or only a few of these units in various proportions. The following table gives the amount of each amino acid, which has been obtained from a few typical natural proteins, expressed in percentages :—

	Salmine.	Globin of Hæmoglobin (Horse).	Serum-albumin.	Serum-globulin.	Excelsin from Brazil Nut.	Caseinogen.	Gelatin.	Silk-fibroin.
Glycine - -		–	0	3·5	0·6	0	16·5	36·0
Alanine - -		4·2	2·7	2·2	2·3	0·9	0·8	21·0
Valine - -	4·3	29·0		+	1·5	1·0	1·0	
Leucine - -			20·0	18·7	8·7	10·5	2·1	1·5
Isoleucine -								
Phenylalanine		4·2	3·1	3·8	3·5	3·2	0·4	1·5
Tyrosine		1·3	2·1	2·5	3·1	4·5	0	10·5
Serine - -	7·8	0·6	0·6			0·2	0·4	1·6
Cystine - -		0·3	2·5	0·7		0·1		
Proline - -	11·0	2·3	1·0	2·8	3·6	3·1	5·2	+
Oxyproline -		1·0				0·3	3·0	
Aspartic acid -		4·4	3·1	2·5	3·8	1·2	0·6	+
Glutamic acid		1·7	7·7	8·5	12·9	11·0	0·9	0
Arginine	87·4	5·4			16·1	4·8	7·6	1·0
Lysine - -	0	4·3			1·6	5·8	2·8	+
Histidine -	0	11·0			1·5	2·6	0·4	+
Tryptophane -		+	+	+	+	1·5	0	

The known proteins are classified according to their origin, solubility, coagulability on heating and other physical properties without strict reference to their chemical composition. This classification is, however, borne out by their actual chemical composition as far as it is known.

The following is the classification adopted by the Chemical and Physiological Societies in 1907:—

1. **Protamines.**

Basic substances, very rich in nitrogen, which occur in ripe fish-sperm combined with nucleic acid as salts. They are soluble in water giving it an alkaline reaction, and form salts with sulphuric acid, etc., e.g. salmine in salmon, clupeine in herring.

2. **Histones.**

Basic substances, less rich in nitrogen than the protamines, which occur in unripe fish sperm, red blood corpuscles, and also in the thymus. They are precipitated from their solutions by the addition of ammonia.

3. **Albumins.**
4. **Globulins.** } These occur together in most tissues and fluids whether of animal or vegetable origin.

Albumins are soluble in water, globulins are insoluble in water but are soluble in dilute solutions of neutral salts. Their solutions are coagulated by heat. Globulins are also distinguished from albumins in that they are more easily precipitated from solution by neutral salts, thus globulins are precipitated by complete saturation of their solution with magnesium sulphate or by half-saturation with ammonium sulphate, e.g. serum-globulin, fibrinogen and its derivative fibrin, myosinogen and its derivative myosin: crystalline vegetable globulins, e.g. edestin in hemp seed, excelsin in Brazil nuts: other vegetable globulins, e.g. legumin in peas, beans; amandin in almonds; conglutin in lupine seeds.

Albumins are not thus precipitated; their separation is only effected when ammonium sulphate is present to complete saturation of the solution, e.g. ovalbumin, serum-albumin, various plant albumins.

5. **Glutelins.**
6. **Gliadins.** } Proteins contained in cereals.

The glutelins are insoluble in water and alcohol but soluble in very dilute alkali or acid, e.g. avenin in oats, glutelins in maize, wheat, rye.

The gliadins are insoluble in water, but soluble in alcohol of seventy to eighty per cent., e.g. wheat-gliadin, rye-gliadin, hordein in barley.

7. **Scleroproteins.** (Formerly albuminoids.)

These constitute the greater part of the skeletal structure of animal organisms. Their properties are very various; they are characterized mainly by their insolubility and stability to the action of acids, enzymes, etc., e.g. keratin from skin, hair, horn, feathers and egg-membranes; collagen (gelatin); elastin; spongin; silk-fibroin; silk-gelatin.

8. Phosphoproteins.

The chief characteristic of these proteins is that they contain
phosphorus, and that they constitute the greater part of the protein
present in the foodstuffs of the embryo-bird and young mammal. They 1
are more acid in character than any of the other proteins, are soluble 2
only in dilute alkalies or alkali carbonates (with which they form soluble
salts liberating carbonic acid) and are precipitated from their solutions 3
by the addition of acid, e.g. caseinogen in milk, vitellin in egg-yolk.

9. Conjugated Proteins.

The protein molecule is here combined with a "prosthetic group," which is not a protein.

(*a*) Nucleoproteins. Protein combined with nucleic acid.

(*b*) Chromoproteins. Protein combined with a chromogenic substance, e.g. hæmoglobin, where protein is combined with hæmatin.

(*c*) Glucoproteins. Protein combined with a carbohydrate, e.g. the mucins.

Note.—The carbohydrate which is obtained in small quantities from coagulable proteins, is apparently not in the form of a prosthetic group, but originates most probably from glucoprotein or carbohydrate which separates out with the protein in its isolation.

10. Derivatives of Proteins.

Proteins are acted upon by acids and alkalies and by enzymes and are converted into

(1) **Metaproteins.** { (*a*) **Acid Albumin.**
(*b*) **Alkali Albumin.** }

(2) **Proteoses.** Formed from all proteins by the further action of acids and alkalies, and by enzymes, e.g. albumose, caseose, globulose, elastose.

(3) **Peptones.** Formed from all proteins by the prolonged action of acids, alkalies, enzymes, e.g. fibrin-peptone, casein-peptone.

(4) **Polypeptides.** The majority are synthetical substances of known composition, e.g. glycyl-glycine, leucyl-tyrosine, etc. Several have now been obtained from proteins by hydrolysis, e.g. glycyl-alanine, leucyl-glutamic acid and a tetrapeptide composed of two molecules of glycine, one molecule of alanine and one molecule of tyrosine.

(5) **Amino Acids.** The metaproteins, the proteoses and the peptones still possess the properties of a protein, but the ultimate products, the amino-acids, do not. The polypeptides are intermediate between the peptones and amino-acids, and in all probability they make up a large proportion of peptone. Changes of this kind occur in digestion, and are necessary before any assimilation of the protein can take place. The protein in this way becomes diffusible and can pass through colloidal membranes.

THE PRINCIPAL REACTIONS OF THE PROTEINS.

The chief reactions of the proteins depend upon :—

1. Their chemical nature. (*a*) constituent radicles (colour reactions). (*b*) alkaloidal (from the manner in which the nitrogen atoms are combined together).

2. Their colloidal nature. (High molecular weight.)

Carry out the following reactions with the egg-white solution and tabulate your results carefully so as to compare with the reactions given by gelatin, hair, serum, derivatives of protein, etc.

Egg-white has been beaten to break up the membranes and filtered through calico. It contains about 10 per cent. protein (chiefly albumin) and has been diluted with nineteen times its volume of water so that the solution contains 0·5 to 1 per cent. protein. Observe the precipitate of ovomucin (formerly regarded as globulin). This passes into solution on adding a little salt solution (NaCl, Am_2SO_4). Egg-white has a faintly alkaline reaction.

A. COLOUR REACTIONS.

1. Biuret reaction.

To the egg-white solution add caustic soda and then, drop by drop, dilute copper sulphate solution (1 per cent.), mixing after each addition: a violet colour appears. This is due to the presence of at least two —CO—NH groups. (See under urea.) Excess of copper sulphate must be avoided as its blue colour masks the reaction.

ee. p. 111.

2. Xanthoproteic reaction.

Heat a little egg-white solution with concentrated nitric acid; a yellow colour is formed; cool, and add ammonia or soda in excess; the colour changes to orange. This is most probably due to the formation of nitro compounds with the aromatic units contained in the protein, namely, tyrosine, phenylalanine. See phenol. benzoic ac. p. 131-2

p. 123

3. Millon's reaction.

To some egg-white solution add Millon's reagent; a white precipitate is formed which becomes red on heating. This is due to the presence of tyrosine in the protein. (Compare oxy-derivatives of benzene.)

p. 129.

Millons reag. Mercury in ~~nitric + nitrous ac.~~ conc. nitric

i. e. Mercuric + Mercurous nitrite + nitrate.

4. Sulphur reaction.

Add a drop of lead acetate solution to some egg-white solution and then caustic soda sufficient to redissolve the precipitate which is first formed. Boil. A brown coloration, sometimes black, occurs: this is due to the separation of hydrogen sulphide from the cystine unit in the protein molecule, which gives lead sulphide with the lead acetate. (Compare p. 107.)

5. Adamkiewicz reaction.

Add excess of glacial acetic acid to the solution, and run in concentrated sulphuric acid to the bottom. On standing, or on gently shaking, a reddish-violet colour appears at the junction of the fluids which gradually spreads throughout the solution.

It has been shown by Hopkins and Cole that this reaction is due to the presence of glyoxylic acid in the glacial acetic acid; it is therefore better to use a solution of glyoxylic acid instead of glacial acetic acid which may not contain this substance (if kept in the dark).

Treat some of the solution of egg-white with a little glyoxylic acid solution and run in concentrated sulphuric acid as before to the bottom of the test tube. The purple ring as above described slowly forms.

This reaction is due to the presence of tryptophane in the protein molecule. Some substance is formed from the glyoxylic acid which reacts with the tryptophane.

Pyrix - amino - propionic.

N – C CH2
C HC – C
N – C – N

A similar colour is produced when commercial sulphuric acid is added to a protein solution containing a minimal quantity of formaldehyde. This reaction is brought about by the presence of oxidizing agents in the sulphuric acid which act upon the formaldehyde (Rosenheim). It is not due to the formation of glyoxylic acid by aldol condensation of formaldehyde and oxidation (Dakin).

formaldehyde? in [illegible]

It should be noted that this reaction has been used for many years for detecting formaldehyde which has been added to milk as a preservative.

Proteins, precipitated by alcohol and washed with ether, give a blue colour when heated with concentrated hydrochloric acid. Liebermann's reaction. A reddish-violet colour, which ultimately becomes brown, is produced on heating proteins with concentrated hydrochloric acid.

These reactions, according to Cole, are due to the presence of tryptophane: in the first, glyoxylic acid is derived from the alcohol and ether; in the second, furfural is formed from carbohydrate in the protein which reacts with the tryptophane.

The green to blue colour produced when proteins are heated with benzaldehyde, a drop of ferric chloride and concentrated hydrochloric acid—Reichl's reaction—is also due to the presence of tryptophane.

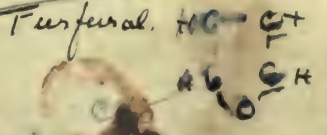
6. Molisch's reaction.

Add a few drops of α-naphthol solution to the egg-white solution, mix thoroughly and then run some concentrated sulphuric acid under the solution. At the junction of the two liquids a violet ring is formed.

This reaction is due to the formation of furfural from the carbohydrate radicle in the protein which combines with tryptophane. (See p. 68.)

phenol ring
in tryptophane
phenol in α naphthol

Furfural.



alternative for:
for glucose

CH2OH do not
CH get up
CHOH
CHOH
CHOH
C HOH.

B. PRECIPITATION REACTIONS.

1. Heat Coagulation.

ll higher
xc. gelatin coag.
y heating in
lightly ac. sol
precipitats
in neutral sol.

Heat some egg-white solution: as the reaction is alkaline, only an opalescence occurs, with perhaps a slight precipitate on the surface of the glass. Faintly acidify another portion with one to two drops of dilute acetic acid and again heat. A cloudiness and then a flocculent precipitate of coagulated protein is formed. This precipitate is not soluble in dilute acids and alkalies in the cold, but it gradually dissolves on heating with caustic soda.

Complete coagulation only occurs when the solution is faintly acid. It is helped by the addition of a little sodium chloride or calcium chloride. If too acid or alkaline, metaprotein is formed and this (see later) is only coagulable in neutral solutions.

Coagulable proteins are separated from other proteins in this way, by adding a little salt, boiling, and whilst boiling, faintly acidifying.

Determine the Temperature of Coagulation.

Faintly acidify a little egg-white solution in a test tube with dilute acetic acid. Immerse the tube in a beaker of water and heat gradually, stirring constantly. Place a thermometer in the egg-white solution and note the temperature at which a cloudiness appears in the solution, and when, on further heating, it separates into flocculi.

2. Alcohol.

Add excess of alcohol to some egg-white solution. A precipitate is formed. This precipitate is at first capable of re-solution in water, but on prolonged contact with alcohol it is rendered insoluble, the protein being coagulated.

(2*a*) Ether. Add to some egg-white solution about half its volume of ether and mix thoroughly by inverting the liquids. A gelatinous solution results, which contains coagulated protein.

3. Strong Mineral Acids.

Heller's test. To some egg-white solution add concentrated nitric acid by means of a pipette, or by gently pouring down the sides of the tube, so that the acid forms a distinct layer below the solution. At the junction of the two liquids a white ring of coagulated protein is formed. Mix the liquids by shaking; the precipitate is only with difficulty soluble in excess of the acid, even on heating.

u.f. proteoses

(3*a*) Metaphosphoric acid. A precipitate is formed on adding a little freshly prepared metaphosphoric acid solution to egg-white solution.

(4) Solutions of the Heavy Metals.

Add two or three drops of mercuric chloride solution to some egg-white solution. A heavy white precipitate of the mercury compound is formed. This dissolves on adding some saturated sodium chloride solution. The mercury compound is reprecipitated from its solution in sodium chloride on adding a few drops of dilute hydrochloric acid.

Copper sulphate, added drop by drop, gives a bluish-violet precipitate which dissolves in caustic soda, giving a violet solution (biuret reaction).

Ferric chloride gives a precipitate soluble in excess.

Lead acetate and basic lead acetate give white precipitates.

alkaloids. complex. nitrogenous-basic. containing pyridine groups. e.g. morphi

5. Precipitation by Alkaloidal Reagents in Acid Solution.

(*a*) Hydroferrocyanic acid. To a little egg-white solution add a few drops of glacial acetic acid, and then, drop by drop, potassium ferrocyanide solution. A voluminous precipitate is formed. This precipitation is less complete in the presence of neutral salts and does not occur in neutral solutions. × not for g

(*b*) Picric acid. A yellowish precipitate is formed on adding picric acid to egg-white solution.

(*c*) Potassio-mercuric iodide. Brücke's reagent. Acidify with dilute hydrochloric acid, and then add a few drops of potassio-mercuric iodide; a whitish precipitate is formed. omit

(*d*) Trichloracetic acid. A white precipitate is formed on adding an equal volume of 10 per cent. trichloracetic acid.

(*e*) Tannic acid. A brownish precipitate is formed. v. ma
prog

(*f*) Bromine water gives a white precipitate.

(*g*) Phosphotungstic acid. A white precipitate is produced when phosphotungstic acid is added to egg-white solution previously acidified with hydrochloric acid.

These reagents are the most commonly employed for removing proteins from solution, e.g. in the preparation of glycogen from the liver, the liver is broken up and thrown into boiling water acidified with acetic acid. The coagulated proteins are filtered off, and from the filtrate the remaining proteins are precipitated by trichloracetic acid or by Brücke's reagent. The filtrate is then free from protein and the glycogen is precipitated by alcohol.

The tannic acid compound is of commercial importance. Leather is made by tanning skins.

The hydroferrocyanic acid reaction is often used clinically for detecting protein ("albumin") in the urine.

Estimation of Protein.

Picric acid is used in the estimation of protein by Esbach's method for clinical purposes. Pour into the Esbach tube (fig. 38) some egg-white solution up to the mark U, and then Esbach's reagent up to the mark R. Cork the tube and mix thoroughly. Allow to stand for twenty-four hours. Read off the height of the deposit in the tube which is graduated in amounts of protein in grammes per litre. This method is often employed in estimating albumin in urine.

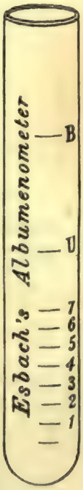


FIG. 38.

Carry out the same reactions described under egg-white with :—

(A) The 1 per cent. solution of gelatin.

v. B. specific.

Gelatin is insoluble in cold water, but dissolves in hot water. On cooling, the solution sets into a jelly. If boiled too long, the power of gelatinization is lost. Dilute gelatin solutions do not set into a jelly on cooling.

N. B.

On tabulating the results and comparing with those obtained with the egg-white solution it will be seen that with gelatin :—
(1) The xanthoproteic reaction is negative or very faint,
(2) The Millon reaction is negative,
(3) The sulphur reaction is negative,
(4) The Adamkiewicz reaction is negative,
from which (1) the paucity of aromatic radicles, (2) the absence of tyrosine, (3) the absence of cystine, (4) the absence of tryptophane in the gelatin molecule may be concluded.

(5) It is not coagulated by heat; it is precipitated, but not coagulated by alcohol, etc.

(6) It is not precipitated by concentrated mineral acids.

? ?

(7) Heavy metals may or may not give a precipitate, thus, e.g. mercuric chloride does not precipitate a dilute gelatin solution when neutral, but on acidifying with hydrochloric acid a large white precipitate is obtained. Lead acetate does not precipitate gelatin.

C.f. proteoses

(8) Hydroferrocyanic acid does not precipitate gelatin, or only a very slight precipitate is formed.

(9) Tannic acid gives a very copious and voluminous precipitate.

Estimate the amount of protein by Esbach's method.

(B) The hair or horn.

With hair or horn, owing to its insolubility, only certain of the colour reactions will be capable of being observed, e.g. the xanthoproteic reaction, Millon's reaction, sulphur reaction.

THE HYDROLYSIS OF PROTEINS.

Proteins are hydrolyzed into their constituent amino acids by boiling with concentrated mineral acids or alkalies for six to ten hours. This hydrolysis of the complex protein takes place in several stages, products intermediate between the large protein molecule and the amino acid molecules being formed. These are metaprotein, proteoses, peptones, polypeptides. In the formation of metaprotein only a comparatively small change in the protein molecule occurs. Proteoses and peptone are formed by the breaking of the large molecule into several large complexes, each of which is gradually broken up into smaller complexes. Finally, the amino acids are formed from these smaller complexes.

A. METAPROTEIN.

Metaprotein is formed by the action of dilute acids and alkalies on proteins fairly rapidly at 60° and higher temperatures, more slowly at 37° C. It is insoluble in water, and in solutions of neutral salts, but it dissolves in dilute acids and alkalies. In acid solution it is known as acid-albumin and in alkaline solution as alkali-albumin. On boiling its solution in dilute acid or alkali it is not precipitated, but when precipitated by neutralization and the solution then boiled it is coagulated.

N.B.

B. THE PROTEOSES AND PEPTONE.

These are obtained by the further action of acid and alkalies on proteins. They are also formed during the digestion of the proteins by the action of the ferments pepsin and trypsin. They are the end-products of the action of pepsin on proteins. Witte's peptone consists of a mixture of these substances, and is made by digesting fibrin with pepsin.

C. POLYPEPTIDES AND AMINO ACIDS.

The presence of polypeptides amongst the hydrolytic products of proteins can only be shown by complex methods of analysis resulting in their actual isolation from the mixture. The same statement applies to the amino acids. Certain of them, however, can be detected in the mixture, e.g. cystine (p. 107), tyrosine (p. 168), tryptophane (pp. 143, 168).

Preparation of Acid-albumin and Alkali-albumin.

To some dilute egg-white solution add a little 0·1 per cent. sulphuric acid or one to two drops of glacial acetic acid, and place it in the water bath at 40-50° C. for fifteen minutes. Acid-albumin is formed.

Add a little dilute caustic soda to some egg-white solution, and heat it in the water bath at 40-50° C. for ten minutes. Alkali-albumin is formed.

(*a*) On boiling the solutions no coagulation occurs.

(*b*) Neutralize carefully a little of the solutions with dilute alkali or acid. Metaprotein is precipitated and is soluble in excess of the acid (or alkali).

Properties of Metaprotein.

Acid-albumin has been prepared by treating egg-white (or serum) with ten times its volume of 0·4 per cent. hydrochloric acid and keeping the mixture in the incubator at 37° C. for at least twenty-four hours.

Alkali-albumin has been prepared by treating egg-white (or serum) with ten times its volume of 0·1 per cent. caustic soda and keeping the mixture in the incubator at 37° C. for about eighteen hours.

Examine these solutions as follows :—

(*a*) Boil a portion of the solutions. No coagulation occurs.

(*b*) Neutralize the remainder of the solutions very carefully with 0·4 per cent. caustic soda and 0·4 per cent. hydrochloric acid respectively. A bulky white precipitate is formed in each case. Filter off the precipitate and wash it once with water. Scrape the precipitate off the filter paper and suspend it in distilled water. Carry out the following reactions with a portion of each of the suspensions :—

1. Add some 0·4 per cent. hydrochloric acid; the precipitate dissolves. Neutralize with dilute sodium carbonate; the precipitate is again thrown down.

2. Add some 0·4 per cent. caustic soda. The precipitate dissolves. On neutralizing with 0·4 per cent. hydrochloric acid the precipitate again comes down.

3. Add some saturated ammonium sulphate solution, drop by drop. The precipitate does not dissolve in any concentration of the salt. (Distinction from globulins.)

globulin is
nsol in wate
- satd salt
solns but sol. in dil. salt. solns

4. Boil a portion of the suspension. Coagulation occurs. This is verified by cooling and adding some 0·4 per cent. hydrochloric acid when the precipitate is found to be no longer soluble.

5. Dissolve in a little 0·4 per cent. hydrochloric acid and boil. No coagulum is formed. Cool, and neutralize carefully with dilute sodium carbonate solution. Acid-albumin is precipitated, which is soluble in dilute hydrochloric acid or caustic soda.

Acid and alkali albumin can also be obtained as follows :—

(*a*) Add to a little undiluted egg-white about one-third of its volume of glacial acetic acid, and mix thoroughly. Allow it to stand : the solution sets to a jelly consisting of acid-albumin. omit.

(*b*) Add to a little undiluted egg-white about one-third of its volume of dilute caustic soda, and mix thoroughly. A jelly consisting of alkali-albumin (Lieberkühn's jelly) forms.

Prove the presence of acid- and alkali-albumin in these jellies by diluting with water, neutralizing and carrying out the above tests for acid- and alkali-albumin.

By the action of the strong acid on the egg-white coagulation of the protein also occurs. Hence the opalescent colour and incomplete solution on diluting with water. Acid-albumin is only formed on prolonged standing.

Properties of Proteoses and Peptone.

Examine the 5 per cent. solution of Witte's peptone which has been dissolved in hot water and filtered from a small quantity of insoluble material.

1. Boil the solution and acidify when boiling with dilute acetic acid. No coagulum is formed. Cf. [illegible] 15

2. Add concentrated nitric acid, drop by drop. A white precipitate is formed, which dissolves on heating, and forms a yellow solution, but reappears on cooling. Specific for proteose? C.f. p. 150. Heller's Test.

3. Add a drop of glacial acetic acid and 2 to 3 drops of potassium ferrocyanide. A white precipitate is formed. This disappears on heating, but reappears on cooling. Hydro-ferrocyanic Test, not by gelatin.

4. Add 1 to 2 drops of copper sulphate solution ; a bluish-white precipitate results.

5. Add lead acetate solution ; a white precipitate is formed. Conf. gelatin.

6. Tannic acid also gives a white precipitate.

7. Apply the colour reactions for proteins. They are all obtained. The colour with the biuret test is less violet and more red in colour.

PROPERTIES OF COLLOIDAL SOLUTIONS.

The fats and soaps, the polysaccharides and proteins, the principal substances with which physiological chemistry has to deal, are colloidal in nature. Their properties depend so much upon this fact that it is necessary to examine the nature of colloidal solutions.

I. Indiffusibility through Membranes.

(*a*) Place some litmus solution with a drop or two of dilute hydrochloric acid in a parchment paper dish and allow it to float in a beaker of distilled water. The litmus does not diffuse out, but the hydrochloric acid passes into the surrounding water. Test for hydrochloric acid by silver nitrate in the presence of nitric acid. If the process of dialysis be continued sufficiently long (repeated changes of water), the red colour will disappear and the litmus will become blue.

(*b*) Perform the same experiment with a mixture of starch solution and glucose; the former being a colloid does not diffuse out, but the latter, a crystalloid, diffuses out and can be tested for in the surrounding water by Trommer's or Fehling's test.

(*c*) Egg-white solution treated in the same way does not diffuse out through a parchment paper membrane. Test the surrounding water for protein by the xanthoproteic, Millon's, and the biuret reactions, and show that protein is absent.

The ovomucin (or globulin) may be precipitated in the paper dish if the egg-white solution be dialyzed long enough, as it is insoluble in distilled water; it dissolves on adding a little salt.

II. Precipitation by Electrolytes.

(*a*) Add a little sodium chloride to some soap solution in small quantities at a time and occasionally shake. The soap is precipitated. Test as under soap (p. 53).

(*b*) To some starch solution add some ammonium sulphate; precipitation of the starch occurs if the solution be occasionally shaken, so as to dissolve the salt. Filter off the starch, suspend it in water and test with iodine solution.

see below (*c*) Saturate some egg-white solution with (1) sodium chloride, (2) magnesium sulphate, by grinding it in a mortar with the salt. The ovomucin (or globulin) is precipitated.

The same result is obtained by half saturating the egg-white solution with ammonium sulphate, i.e. by adding an equal volume of saturated ammonium sulphate solution. Filter off the ovomucin and test it for protein. Saturate the filtrate with finely powdered ammonium sulphate crystals. The egg-albumin is precipitated. Filter off the precipitate, dissolve it in water and boil after adding a drop of acetic acid. The egg-albumin is coagulated. This method is employed for separating ovomucin and globulins, which are less soluble, from albumins, which are more soluble and are only precipitated from solution by completely saturating with ammonium sulphate. (See under separation of proteins, p. 159.)

Other properties of colloidal solutions are:—

III. Low osmotic pressure as measured directly, or by their freezing and boiling-points.

IV. Impossibility, or great difficulty, in making the substance crystallize from solution. It may, in fact, be stated that the solvent must be in some way changed (as by adding alcohol or ammonium sulphate) before crystallization can occur.

Proteins have been prepared in a crystalline state in this way. Of all the proteins the vegetable globulins are most easily obtained as crystals. Edestin and excelsin can be prepared by extracting the oil-free seeds with 10 per cent. sodium chloride solution warmed to 60°, filtering and allowing to cool, when crystals separate out.

From egg-white or serum the crystalline albumin is prepared by half-saturating it with ammonium sulphate, filtering off the globulin, and acidifying the filtrate with acetic acid until there is a slight permanent precipitate. On standing for twenty-four hours, the precipitate increases in amount and on examination with a microscope is found to consist of acicular crystals.

The whole of the albumin is, however, not obtained in a crystalline form; the remainder has been termed conalbumin by T. B. Osborne.

Hæmoglobin crystallizes out when a solution of hæmoglobin is mixed with alcohol and allowed to stand.

V. Tendency to form jellies.

Evaporate a little gelatin or peptone solution to dryness on the water bath and note that a horny gelatinous mass remains.

Fine suspensions of solid matter in a liquid resemble colloidal solutions in many respects, e.g. arsenious sulphide, kaolin.

1. They are opalescent, as colloidal solutions often are, e.g. starch, glycogen.

2. They pass through filter paper, but not through a parchment membrane.

Filter some arsenious sulphide and kaolin suspensions through paper, and note that they pass through.

3. They may be precipitated by electrolytes.

Add some salt to the arsenious sulphide and kaolin suspensions; shake thoroughly and note that precipitation occurs.

Colloidal solutions of inorganic salts may be readily obtained by dissolving them in glycerol and then throwing into water. (Craw.)

Dissolve a little potassium chromate and lead nitrate, separately, in glycerol; mix the two solutions and pour the mixture into water. Colloidal lead chromate is formed. Compare the result with the mixture of the two substances dissolved in water. Lead chromate is precipitated.

THE FORMATION OF EMULSIONS.

Permanent emulsions are formed when a solution containing a colloid is shaken up with another liquid not miscible with it, and separation of colloidal particles, insoluble in either of the media, occurs at the same time.

The most typical permanent emulsions are observed with fats. The fats are neutral substances, but generally they contain a little fatty acid, which gives them an acid reaction and causes the formation of soap when shaken up with alkali, which is insoluble in the fat.

1. **Detection of Fatty Acid in Fat.** To a little alcohol in a test tube add a drop of phenolphthalein and then a drop or two of *very dilute* alkali, just enough to produce a red colour. Now add a little fat or olive oil dissolved in alcohol or ether. If fatty acid be present the red colour disappears.

Compare with neutral olive oil which has been prepared by dissolving it in ether, shaking up with dilute sodium carbonate solution, washing free from alkali, and finally distilling off the ether.

2. **Formation of Emulsions.** Take five test tubes and in them place

(1)	(2)	(3)	(4)	(5)
10 c.c. H_2O	10 c.c. H_2O	10 c.c. H_2O	10 c.c. H_2O	10 c.c. H_2O
2 c.c. neutral olive oil.	1 drop 8 °/₀ NaOH	2 drops oleic acid	1 drop 8 °/₀ NaOH	1 drop 8 °/₀ NaOH
	2 c.c. neutral olive oil.	2 c.c. neutral olive oil.	2 drops oleic acid	2 c.c. ordinary olive oil.
			2 c.c. neutral olive oil.	

Shake each thoroughly. Only in (4) and (5) is a permanent emulsion formed, separation occurring in (1), (2) and (3) after a short time. (5) shows that ordinary fat contains free fatty acid.

The same result can also be seen by dropping a little neutral olive oil and a little ordinary olive oil on to the surface of some dilute sodium carbonate solution in watch glasses. The neutral oil drop remains clear, whilst the ordinary oil drop spreads out and gives a milky emulsion.

egg albumen
Soap
both
lowers S.T.

The formation of emulsions is due to the fact that a layer of soap, which is insoluble in the fat, is made round the fat particle. A layer of oil, which is insoluble in the water, may also form round the soap particle.

In the same way an emulsion is obtained when oil or petroleum is shaken up with egg-albumin. In both cases a layer of coagulated egg-albumin is formed round each particle.

To prove this, shake up a little egg-albumin solution in a test-tube; a fine layer of mechanically coagulated egg-albumin will be seen to be formed which rises to the surface on standing.

Protein solutions have free-surface coatings, and by mechanically shaking, these are heaped up to form solid masses of protein. The following simple experiment demonstrates the surface coating of a protein solution :—

Take two beakers; in the first place clean water, in the second place egg-white solution. Float on to the surface of each a magnetized needle and observe what happens when a magnet is brought near. In the first, the needle spins round; in the second, only a slight attraction or repulsion is seen. If the beaker be suspended by a wire, in the latter case the whole beaker would swing round, whereas in the former only the needle would rotate. (Ramsden.)

SEPARATION OF PROTEINS.

Proteins may be separated in unchanged condition from their solution and from one another by making use of their colloidal property of precipitation by electrolytes. In general it may be said that those proteins which are more complex and have a higher molecular weight are more easily thrown out of solution than the less complex.

A very large number of salts have been used as electrolytes for this purpose, the principal ones being sodium chloride, magnesium sulphate and ammonium sulphate. Of recent years ammonium sulphate has been almost exclusively used as by its means the solutions can be fractionally precipitated. Saturation with sodium chloride or magnesium sulphate generally throws down the same substance as half-saturation with ammonium sulphate. Complete saturation with ammonium sulphate completely precipitates globulins and albumins and in faintly acid solution all proteoses are precipitated. Peptone cannot be salted out and remains in solution after all other proteins have been separated.

The following table, when completed, will give a summary of the effect of the various salts used in the separation of proteins :—

	Sodium Chloride.	Magnesium Sulphate.	Ammonium Sulphate.	
	Saturation.	Saturation.	Half-Saturation.	Saturation.
Egg white - -	globulin	globulin	globulin	glob + albumin
Gelatin - - -	no pptc	pptc	pptc	Small pptc
Blood serum (p. 211)	Globulin	globulin	globulin	glob + albumin
Acid albumin -	pptc	pptc	pptc.	
Alkali albumin -	no pptc	pptc	pptc	
Witte peptone -	prim prot.	prim. prot.	prim prot	prim + second. proteose (peptone in sol)

globulins ppte Sat. Sod. chlorid.
mag. Sulphate
$\frac{1}{2}$ Sat am. sulphate.
albumins Sat " "

Egg-white.

(*a*) **Sodium Chloride.** Gradually add finely powdered sodium chloride to some of the egg-white solution until the solution is saturated,[1] i.e. until no more is dissolved and crystals remain. The globulin is precipitated. Filter, heat a portion of the filtrate to boiling and faintly acidify with dilute acetic acid. The ovalbumin is precipitated. It is also precipitated by adding a few drops of dilute hydrochloric acid.

(*b*) **Magnesium Sulphate.** In the same way saturate the egg-white solution with magnesium sulphate crystals.[2] The globulin is again precipitated. Test the filtrate as above for ovalbumin.

(*c*) **Ammonium Sulphate.** Add an equal volume of saturated ammonium sulphate solution to some egg-white solution, thus making the solution half saturated. Mix. The globulin is precipitated. Filter. Saturate the filtrate with ammonium sulphate crystals.[3] The ovalbumin is precipitated.

Gelatin.

(*a*) **Sodium Chloride.** Saturate a portion of the gelatin solution with finely powdered crystals of sodium chloride.

(*b*) **Magnesium Sulphate.** Perform the same operation with magnesium sulphate. The gelatin is precipitated.

(*c*) **Ammonium Sulphate.** Half saturate some of the gelatin solution by adding an equal volume of saturated ammonium sulphate solution. The gelatin is precipitated.

A further small precipitate may be obtained on saturating the filtrate with crystals of ammonium sulphate. This is most probably impurity in the gelatin.

Metaprotein.

like globulin

1. **Acid-albumin.** Acid-albumin is precipitated completely on saturating the solution with (*a*) sodium chloride, (*b*) magnesium sulphate, or (*c*) by half-saturating with ammonium sulphate.

" except

2. **Alkali-albumin.** Alkali-albumin is not separated from solution by saturation with (*a*) sodium chloride, but is precipitated by saturation with (*b*) magnesium sulphate, or by half-saturation with (*c*) ammonium sulphate. Prove by testing the filtrate by means of the protein reactions.

Proteoses and Peptone.

(*a*) **Sodium Chloride.** Saturate a portion of the Witte peptone solution with crystals of sodium chloride. A precipitate consisting of primary proteoses is obtained. The filtrate contains the secondary proteoses which give the protein reactions.

(*b*) **Magnesium Sulphate.** The same result is obtained when Witte peptone solution is saturated with magnesium sulphate. Prove that the filtrate still contains protein.

---

[1] 3·6 gm. of sodium chloride to every 10 c c. of solution.

[2] 10·2 gm. of cryst. magnesium sulphate to every 10 c.c. of solution.

[3] 4·0 gm. of ammonium sulphate to every 10 c.c. of half-saturated solution.

(*c*) **Ammonium Sulphate.** Add to 20 or 25 c.c. of the Witte peptone solution an exactly equal volume of saturated ammonium sulphate solution (=half-saturation). A white precipitate of **primary proteoses** is formed. If the mixture be well stirred with a glass rod, which is best covered at one end with a piece of rubber tubing, the precipitate will gather together and can be almost completely collected as a sticky mass on the end of the rod. Remove it in this way or by filtering. Dissolve the mass in a little hot water, and cool the solution. With a small portion perform the following protein reactions as described above :—

1. With acetic acid and potassium ferrocyanide.
2. With concentrated nitric acid.
3. With copper sulphate.

} All are positive. Primary
" " negative Secondar

This precipitate of primary proteoses consists of two proteoses, proto-proteose and hetero-proteose. These can be separated from one another by dialysis, the hetero-proteose becoming insoluble in water and being precipitated, whilst the proto-proteose remains in solution. They are more easily separated by means of alcohol: hetero-proteose is precipitated by 32 per cent. alcohol; proto-proteose is soluble in alcohol up to 80 per cent. Thus :—

like glob
Salt prote

To the above solution of primary proteose add an equal volume of alcohol. The hetero-proteose is precipitated.

To the liquid remaining after separating the primary proteoses add a drop of dilute sulphuric acid and then finely powdered ammonium sulphate until the solution is saturated. Stir the mixture whilst it is being saturated. A flocculent precipitate of **secondary proteoses** comes down. Collect this on a rod, as before, or filter. Dissolve this precipitate in water and carry out the same reactions 1. 2. 3. as with the primary proteoses. They are negative.

This precipitate, according to Pick, contains three proteoses, but they are difficult to separate; one of them is precipitated by two-thirds saturation with ammonium sulphate.

Peptone remains in solution after the secondary proteoses have been removed by complete saturation with ammonium sulphate, and filtered off.

like
albume
gives
biuret
like pept
hydrolyse
yield ami
acids (th
come fro
proteins

Its chief characteristic is the biuret reaction. Treat a portion of the solution with an excess of strong caustic soda (40 per cent. or solid substance) and add a drop or two of 1 per cent. copper sulphate solution. A pink colour appears. This colour is characteristic of peptone. It is necessary to add a large excess of caustic soda when ammonium sulphate is present in order to decompose it, and in order that the alkalinity is due to sodium hydrate; the alkalinity of the ammonia is not able to produce the colour.

Apply the other colour reactions for proteins to this solution.

Nitric acid gives no precipitate, but, on heating, the colour becomes more or less yellow, changing to orange on making alkaline.

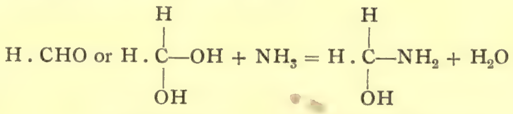
Peptone is precipitated by some of the precipitating reagents for proteins, e.g. tannic acid, lead acetate.

ORIGIN OF PROTEINS.

The natural carbon compounds containing nitrogen belong, as we have seen, to the following groups: amides, amines, amino acids, proteins, ureides, purines, guanidines, heterocyclic compounds. We have to determine their evolution, their changes in plants and animals, and their final degradation into their elements.

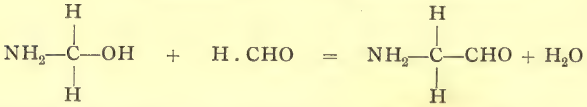
The carbon dioxide in the atmosphere and the ammonia and salts in the soil are the products from which the life-cycle commences, and the plant is the synthesizing agent. The carbon dioxide is converted into formaldehyde. Formaldehyde and ammonia are therefore most probably the initial substances.

Aldehydes combine with ammonia giving aldehyde ammonia: under the influence of alkali they undergo aldol condensation. These two processes probably go on side by side in the plant under the influence of enzymes, as also a simultaneous oxidation and reduction process. Formaldehyde and ammonia will give formaldehyde ammonia: by aldol condensation of this compound with formaldehyde and subsequent oxidation glycine might arise:—



$$\mathrm{H.CHO\ or\ H.}\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\mathrm{—OH + NH_3 = H.}\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\mathrm{—NH_2 + H_2O}$$

Formaldehyde Formaldehyde ammonia



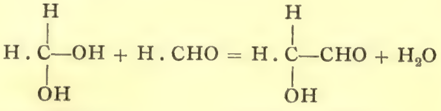
$$\mathrm{NH_2—}\overset{\mathrm{H}}{\underset{\mathrm{H}}{\mathrm{C}}}\mathrm{—OH \quad + \quad H.CHO \quad = \quad NH_2—}\overset{\mathrm{H}}{\underset{\mathrm{H}}{\mathrm{C}}}\mathrm{—CHO + H_2O}$$

Formaldehyde ammonia Formaldehyde Amino-acetaldehyde

$$\mathrm{NH_2.CH_2.CHO + O = NH_2.CH_2.COOH}$$

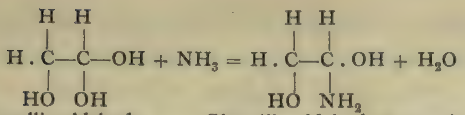
Amino-acetaldehyde Amino-acetic acid or glycine

The aldol condensation of two molecules of formaldehyde will give glycollic aldehyde. The formation of glycollic aldehyde ammonia and condensation with formaldehyde will give an aldehyde compound which on oxidation will pass into serine:—



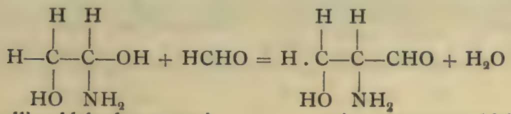
$$\mathrm{H.}\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\mathrm{—OH + H.CHO = H.}\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}\mathrm{—CHO + H_2O}$$

Formaldehyde Glycollic aldehyde



$$\mathrm{H}.\underset{\mathrm{HO}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\mathrm{OH} + \mathrm{NH_3} = \mathrm{H}.\underset{\mathrm{HO}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{NH_2}}{\overset{\mathrm{H}}{\mathrm{C}}}.\mathrm{OH} + \mathrm{H_2O}$$

Glycollic aldehyde Glycollic aldehyde-ammonia



$$\mathrm{H}-\underset{\mathrm{HO}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{NH_2}}{\overset{\mathrm{H}}{\mathrm{C}}}-\mathrm{OH} + \mathrm{HCHO} = \mathrm{H}.\underset{\mathrm{HO}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{NH_2}}{\overset{\mathrm{H}}{\mathrm{C}}}-\mathrm{CHO} + \mathrm{H_2O}$$

Glycollic aldehyde-ammonia α-amino-β-oxypropaldehyde

$$\mathrm{CH_2OH.CHNH_2.CHO + O = CH_2OH.CHNH_2.COOH}$$

α-amino-β-oxypropaldehyde α-amino-β-oxypropionic acid or serine.

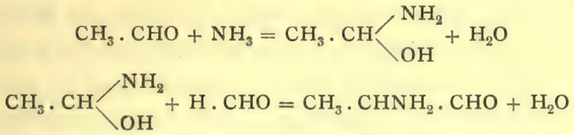
If reduction of the primary alcohol group occurred at the same time as the oxidation of the aldehyde group, alanine would be formed :—

$$\mathrm{CH_2OH.CH.NH_2.CHO = CH_3.CH.NH_2.COOH.}$$

α-amino-propionic acid or alanine.

The presentation of sulphur as hydrogen sulphide at the same time as ammonia to the glycollic aldehyde might result in the replacement of the OH group by the SH group and subsequently cysteine (p. 102) would result. Hence cystine by oxidation.

Alanine might also be formed from acetaldehyde (from carbohydrate) ammonia and formaldehyde by the same process :—



$$\mathrm{CH_3.CHO + NH_3 = CH_3.CH}\Big\langle\begin{matrix}\mathrm{NH_2}\\ \mathrm{OH}\end{matrix} + \mathrm{H_2O}$$

$$\mathrm{CH_3.CH}\Big\langle\begin{matrix}\mathrm{NH_2}\\ \mathrm{OH}\end{matrix} + \mathrm{H.CHO = CH_3.CHNH_2.CHO + H_2O}$$

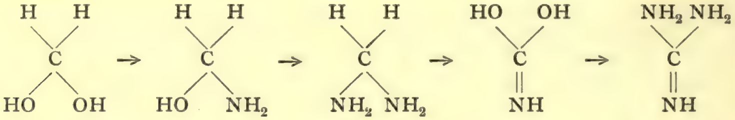
$$\mathrm{CH_3.CHNH_2.CHO + O = CH_3.CHNH_2.COOH}$$

The formulation of a process by which the other amino acids are formed from these products is more difficult; in the absence of our knowledge of these stages further suppositions are of little value.

Fatty acids are formed from carbohydrate: in their oxidation aldehydes may arise which may combine with ammonia and yield an amino acid.

There is still another possibility by which amino acids may arise. Hydrogen cyanide is constantly found in plants, and it is formed in the oxidation of glycine by potassium bichromate. Aldehyde ammonia and hydrogen cyanide combine to form the aminocyanohydrin (p. 103); hydrolysis of this compound will give the corresponding amino acid.

In the formation of formaldehyde ammonia, the NH_2 group replaces an OH group; the replacement of the second OH group by NH_2 would give two NH_2 groups from which an imino group ($= NH$) would be formed. Oxidation of this compound would give two hydroxyl groups again replaceable by NH_2 groups. Guanidine would thus result. It might also be formed directly from carbonic acid and ammonia:—



The combination of the amino acids together will give a protein.

Some of the amines have been proved to be derived from amino acids by loss of carbon dioxide: the same may be the case with the others.

Nothing is known of the origin of the aromatic ring in nature. Possibly inositol is the initial compound, for we know that it is formed by the hydrolysis of phytin, a compound found in green peas, beans, etc., which is a condensation product of two molecules of formaldehyde with phosphoric acid.

An experiment by Windaus is suggestive of the origin of the iminazole and purine rings. By the action of ammonia upon glucose in the presence of light methylglyoxal was found amongst the products. Condensation of this compound with one molecule of formaldehyde and two of ammonia may give methyliminazole; the subsequent oxidation of this compound and condensation again with ammonia and formaldehyde may give xanthine.

The origin of creatine and the pyrrole ring compounds may also be formulated on several plans.

ACTION OF ENZYMES II.

Our knowledge of the changes of the proteins in plants and animals is in comparison with our knowledge of their evolution somewhat less limited. In any translocation of protein from one region to another region hydrolysis of the molecule into amino acids takes place; the amino acids circulate and are built up by the cells requiring them into protein matter.

The hydrolysis of the protein molecule is effected by the proteoclastic or proteolytic enzymes: in animals the proteoclastic enzymes are of two kinds—peptic and tryptic: in plants the proteoclastic enzymes are tryptic in their action. The peptic enzyme acts in an acid medium (stomach) and yields proteoses and peptone; the tryptic enzyme of the pancreas acts in an alkaline medium, that of other organs in alkaline or acid media and yields amino acids. The plant enzyme acts in either an acid or alkaline medium and yields amino acids.

The degradative change of the amino acids has been seen to be of two kinds: either carbon dioxide or ammonia can be split off.

In the first case amines are formed, e.g. putrescine, oxyphenylethylamine; in the second case, a fatty acid will result, e.g. oxyphenylpropionic acid. In animals the main degradative change consists in the simultaneous separation of carbon dioxide and ammonia; an aldehyde may result which on reduction gives an alcohol (e.g. fusel oil) and on oxidation a fatty acid, both of which will break down by oxidation in the same way as the fatty acids. The ammonia and carbon dioxide pass to the liver where they are combined to form urea and this is excreted. Urea has not yet been found in plants, but the plant may use the ammonia and carbon dioxide again for synthesis.

Pepsin is the active principle of the gastric juice and is obtained by extracting the mucous membrane of the stomach with glycerol. It acts upon the proteins in acid solution giving rise to metaprotein, proteoses and peptone.

Their relative proportions will depend upon the strength of the pepsin, and the duration of its action.

It was considered by the earlier workers, Kühne, Chittenden, and Neumeister, that the primary proteoses were the earlier products, but it has been shown by Pick and by Zunz in Hofmeister's laboratory that secondary proteoses may arise quite early in peptic digestion; a small amount of peptone was also noticeable in the early stages. The terms **primary** and **secondary** must therefore not be taken as being primarily and secondarily formed in digestion, but as meaning that they are salted out first and second with ammonium sulphate.

Trypsin is the activated enzyme of the pancreas (p. 194). It acts best in a slightly alkaline solution—0·2 to 0·5 per cent. sodium carbonate. It acts very slowly in a neutral solution, and in a slightly acid (mineral acid) solution it has scarcely any action.

It is obtained by extracting minced pancreas with glycerol. Benger's "liquor pancreaticus" is a commercial preparation obtained in this way; it often contains a sediment consisting of tyrosine. There are numerous other commercial preparations. "Holadin" of Messrs. Fairchild Bros. & Foster is a very active preparation and it contains also lipase and diastase.

The other tryptic enzymes of the animal body are grouped together under the term **erepsin.** Erepsin was the name originally given by Cohnheim to the proteoclastic enzyme of the intestine, which acts on proteoses and peptones yielding amino acids.

Papain is a vegetable proteolytic enzyme obtained from the juice of the papaw tree; it can act both in acid and alkaline media and yields amino acids as products.

Pine-apple juice contains a very active enzyme—bromelin.

The oxidation of the various compounds in plants and animals is carried out by enzymes, which are grouped together under the term **oxidases.** Two classes are distinguished:—

1. Peroxidases, which act upon peroxides, either hydrogen peroxide or an organic peroxide, yielding "active" oxygen.
2. Catalases, which act upon hydrogen peroxide yielding molecular oxygen.

An oxidase is a mixture of a peroxidase and an organic peroxide.

1. Investigation of the Action of Pepsin.

Place a piece of fibrin in a test tube, add 5 c.c. of water and 1 c.c. of pepsin solution.

Into a second test tube, place a piece of fibrin and add 5 c.c. of dilute hydrochloric acid (0·4 per cent., the strength occurring in the stomach).

Into a third test tube, place a piece of fibrin, add 5 c.c. of 0·4 per cent. hydrochloric acid and 1 c.c. of pepsin solution.

Into a fourth tube, place the same as in the third tube, but add boiled pepsin solution.

Into a fifth test tube, place a piece of fibrin, 5 c.c. of 0·4 per cent. alkali and 1 c.c. of pepsin solution.

Put the five test tubes in the water bath at 35 to 40° C. and observe what happens in each tube.

Pepsin will only act in the presence of acid; consequently digestion or solution of the fibrin will only take place in the third test tube, where, in about half an hour, the fibrin will have disappeared. In order to show that it is not the acid which has this effect, the second tube, containing no pepsin but only acid, was used as a control. In this tube the fibrin will have become swollen, but not dissolved. The first, fourth, and fifth tubes will be seen to be unaltered. They both contained pepsin, and they show that pepsin will not act in a neutral or alkaline medium. Hence pepsin only acts in the presence of acid.

Action of Alkali on Pepsin.

Pepsin is destroyed by the action of dilute alkaline solutions, such as are found in the intestine, where the action of pepsin ceases for this reason.

To 5 c.c. of pepsin solution add 2 c.c. of dilute sodium carbonate solution and put it in a water bath at 40° C. for at least half an hour. Then neutralize with 0·4 per cent. hydrochloric acid, add an equal volume of 0·4 per cent. hydrochloric acid and a piece of fibrin, and again keep at 40° C. Digestion will not occur.

Temperature of Destruction of Pepsin.

Take three test tubes and into each place 5 c.c. of water and 1 c.c. of pepsin solution. Heat one of these to 50° C., another to 60° C., and the third to 70° C. for some minutes. Then cool and add to each an equal volume of 0·4 per cent. hydrochloric acid and a piece of fibrin. Place them in the water bath at 40° C. Note in which tube the fibrin is not dissolved by the heated pepsin solution.

The Products of the Action of Pepsin.

Digest about 2 grammes of fibrin with 5 c.c. of pepsin solution in the presence of 0·4 per cent. hydrochloric acid at 40° C. for at least half an hour. Then neutralize the solution with dilute alkali; filter off the metaprotein. Boil the filtrate, and whilst boiling faintly acidify: this destroys the enzyme and removes any unchanged coagulable protein (apparently formed from the fibrin during digestion). Filter.

Test a portion of the filtrate for proteoses with nitric acid. A precipitate is formed, which dissolves on heating and reappears on cooling.

Specifi
for Proteo

Saturate the remainder of the filtrate with ammonium sulphate and filter off the proteoses; test for peptone in the filtrate by the biuret reaction. (*Note.*—Use 40 per cent. or solid soda, see page 161.)

2. Investigation of the Action of Trypsin.

Just as in the case of pepsin, the solution of fibrin by the enzyme is the most ready means of investigating the presence and action of a trypsin.

Place a piece of fibrin in a test tube, add 5 c.c. of trypsin solution, and 5 c.c. of 0·5 per cent. sodium carbonate solution.

Into a second test tube, place a piece of fibrin, 5 c.c. of trypsin solution, and 5 c.c. of water.

Into a third test tube, place a piece of fibrin, 5 c.c. of trypsin solution, and 5 c.c. of 0·4 per cent. hydrochloric acid.

Into a fourth test tube, place a piece of fibrin, 5 c.c. of 0·5 per cent. sodium carbonate solution, and 5 c.c. of boiled trypsin solution to act as a control, in order to show that a ferment is present in the solution in the first test tube.

Put the four test tubes in the water bath at 35 to 40° C. and observe them carefully; only in the tube containing alkali will any change be seen. It will be found that the fibrin has been dissolved without the swelling, which will be observed in the tube containing the hydrochloric acid. The neutral solution and the boiled solution will show no change.

The Products of the Action of Trypsin.

About 200 gm. of caseinogen have been digested with trypsin in about 2 litres of water containing 4 c.c. of ammonia of sp. gr. ·880 at 37° C. for some days, a small quantity of toluene having been added to prevent putrefaction or bacterial decomposition.

1. Examine a portion of the digest. It contains a white precipitate consisting mainly of tyrosine. Filter this off, dissolve a portion in dilute hydrochloric acid and test with Millon's reagent. The solution becomes red.

2. Acidify about 5 c.c. of the filtrate with acetic acid and add bromine water drop by drop; a reddish colour appears which gradually deepens and then disappears as more bromine water is added. When the colour is no longer intensified by adding bromine add 2 to 3 c.c. of amyl alcohol and shake. On standing the amyl alcohol separates coloured red or violet. This reaction was known as the proteinochromogen reaction and is due to the presence of tryptophane, as was shown by Hopkins and Cole.

See p. 143.
Tryptoph.
lee pres. in
free state.
trypsin
digestion proceeds
to amino ac

3. Neutralize the remainder of the filtrate with ammonia and evaporate down to a small volume on the water bath. Allow the residue to stand about twenty-four hours. A crystalline crust forms. Examine the crystals under the microscope—they consist of feathery masses and sheaves of fine white needles, characteristic of tyrosine (p. 134). Filter off the crystals, suspend some in water and test with Millon's reagent. The liquid becomes red on heating.

4. Evaporate the filtrate still further. Leucine and glutamic acid separate out on standing. When examined microscopically the precipitate is found to consist mainly of rounded cones with a radiating striation. When free from tyrosine it does not give Millon's test. Dissolve some in water, add a *little* caustic soda and then dilute copper sulphate drop by drop, shaking after each addition. The cupric hydrate is dissolved, giving a blue solution. On heating, no reduction occurs. The copper salts of leucine and glutamic acid are formed.

3. Investigation of the Action of Papain.

Place a piece of fibrin in a test tube, add 5 c.c. of water and 1 c.c. of papain solution. omit.

In a second test tube, place a piece of fibrin, 5 c.c. of water, 5 c.c. of 0·4 per cent. hydrochloric acid, and 1 c.c. of papain solution.

In a third test tube, place a piece of fibrin, 5 c.c. of water, 5 c.c. of 0·5 per cent. sodium carbonate solution, and 1 c.c. of papain solution.

Repeat the above, but place 1 c.c. of boiled papain solution in the test tubes.

Put the tubes in a water bath at 40° C. and note that the fibrin is dissolved fairly rapidly in the tubes containing acid and alkali, very slowly or not at all in the tube containing water, and not at all in the tubes containing boiled enzyme solution.

4. Investigation of the Action of Oxidases.

These enzymes are very readily detected; a catalase acting upon hydrogen peroxide causes an evolution of oxygen, which does not turn tincture of guaiacum blue; a peroxidase acting upon a peroxide does not cause an evolution of oxygen, but it blues tincture of guaiacum.

I. To a small piece of liver in a test tube add some dilute hydrogen peroxide solution. Notice the evolution of oxygen. a catalase.

II. To a freshly cut surface of a potato apply a drop of tincture of guaiacum. In a short time it becomes blue. must contain both peroxidase & peroxide.

(Compare peroxidase in milk and blood, pp. 173, 213).

Peroxidase - In contact with some sub.e wh. yields ox. readily [illegible] frees ox.

Oxidase is a peroxidase wh. itself contains as sub.ce wh. gives up ox. readily (taking the place of the peroxide in

COMPOSITION OF THE COMMONER TISSUES. FOODSTUFFS.

The principal constituents of all animal and vegetable tissues consist of water, proteins, carbohydrates, fats, and salts. In addition, various other substances are present, which are grouped together under the term "extractives". Amongst the extractives are found the decomposition products of the above three classes of compounds, viz. amino acids, glucose, purines, etc.

These tissues form the foodstuffs of young plants and animals. In the case of the plant the proportions of each compound present in the seed suffice for the maintenance of its early life; its later life is maintained by the synthesis of the compounds from carbon dioxide, ammonia, etc.

In the case of the animal, the constituents of eggs and of milk can maintain the early life, but no single foodstuff contains the proportion of these substances which are necessary to maintain life in an adult; consequently a mixed diet is almost universally taken. The standard diet for man, as given by Voit, consists of:—

protein 110 gm.
carbohydrate 240 ,,
fat 100 ,,

though smaller quantities of protein, as shown by Chittenden, can be taken without loss of body weight and with perfect maintenance of health. Under these conditions the caloric value of the food has to be taken very seriously into account.

In considering the protein, reference should also be made to the relative amounts of the constituent amino acids.

Animal tissues consist mainly of protein with fat and only traces of carbohydrates. In vegetable tissues the amount of carbohydrate preponderates, and the amount of protein is small. Protein is present in greatest amount in the legumes; cereals also contain fairly large quantities. Fruits and green leaves consist mainly of water, nuts almost entirely of protein and fat (oil). The following table shows the proportion of the various constituents in the commoner tissues or foodstuffs :—

	Water.	Protein.	Fat.	Carbohydrate.		Mineral Matter.
				Digestible.	Cellulose.	
Lean meat	77	21	1·5	0·3	—	1·3
Eggs	74	13	12	—	—	1·1
Cheese	36-60	25-33	7·30	3-7	—	3-4
Cow's milk	88	3·4	3·2	4·8	—	0·7
Human milk	90	2·0	3·1	5·0	—	0·2
Wheat flour	13	10	1	75	0·3	0·5
Peas, beans, etc.	15	25	2	50	5	3
Potatoes	75	2	0·2	21	1	1
Fruit	84	0·5	—	10	4	0·5
Nuts (average)	5	20	60	10	—	1

A. MILK.

The principal constituents of milk are:—

Proteins.	(1) Caseinogen. (2) Lactoglobulin. (3) Lactalbumin.
Carbohydrates.	Lactose.
Fats.	Butter Fat.
Salts.	Calcium phosphate, etc.

B. BUTTER.

Butter is obtained from cream by churning. It consists of the triglycerides of palmitic, stearic and oleic acids like other fats, but contains in addition the triglycerides of butyric acid and caproic acid (tributyrin, tricaproin). Recent work by Caldwell and Hurtley points to the absence of tributyrin in butter. When it becomes rancid by the action of bacteria—which contain the enzyme lipase, the smell of butyric acid [?] and caproic acid is noticeable. The presence of these lower volatile fatty acids constitutes the chief difference between butter and ordinary fat.

Margarine consists essentially of mutton fat and beef fat. Butter is sometimes added to it.

C. CHEESE.

Cheese is a product obtained by the action of the rennet ferment upon milk. When rennet is added to milk, which is kept in a warm place, the milk clots. On standing the clot contracts and squeezes out a fluid, whey. Then, by pressure, the remainder of the whey is squeezed out and the clot becomes a hard mass, and consists of casein and fat. If made from skimmed milk, the amount of fat will be small. By bacterial and mould action the cheese then assumes its several varieties. The whey contains the other proteins of the milk together with a globulin [?] formed from the caseinogen by the action of the rennet, lactose, phosphates, etc.

Examination of Milk.

1. Examine a drop of milk under the high power of the microscope. Observe the fat globules of varying size, some of which exhibit Brownian movement.

2. The specific gravity varies from 1028 to 1034. Skimmed milk has a higher specific gravity from 1033 to 1037 owing to the removal of the fat, which is the lightest constituent and floats to the surface as cream.

3. The reaction to litmus is generally faintly alkaline.

4. Boil some milk in a test tube. No coagulation occurs, but a skin gradually forms on the surface. This is most probably due to evaporation at the surface, a layer of the dried constituents forming there and separating out; it may be partly due to the coagulation of the globulin and albumin.

5. Fresh milk gives the *Guaiac Reaction*. Add a little tincture of guaiacum to some fresh milk and then a little hydrogen peroxide. On mixing, the solution gradually becomes blue owing to the oxidation of the guaiacum by an oxidizing enzyme, or oxidase, in the milk (compare blood, *see later*). Instead of hydrogen peroxide, old oil of turpentine, which contains a peroxide, may be used. The reaction generally succeeds better with this than with the hydrogen peroxide.

This reaction is not given by boiled milk, as the oxidase is destroyed by heat, and is often used for distinguishing between fresh and boiled milk. Boil some milk, cool thoroughly, and again carry out the reaction.

6. Extract the fat by shaking up some milk in a test tube with twice its volume of ether. Pour off the ethereal solution into a basin and allow the ether to evaporate. The fat is left behind.

The opacity of the milk is hardly altered. Add a little caustic soda to some milk and again shake up with ether. The solution becomes translucent. The effect of the caustic soda has been[1] partly to destroy the emulsion (fat, protein, *see emulsification before*) and [2] partly to convert the caseinogen, which exists in milk as the calcium salt and forms an opalescent solution, into the sodium salt which gives a clear solution.

1. here depen
on 2. the dea
3. colloidal
sol : mak
suspension m
diff :

7. Heat some milk with a little caustic soda. It turns yellow, then brown, due to the action of the alkali on the lactose. Caramelisation

Separation of the Constituents of Milk.

1. Treat 5 c.c. of milk with 5 c.c. of saturated ammonium sulphate solution ($=\frac{1}{2}$ saturation). The caseinogen is precipitated as the calcium salt, and entangles the fat with it. The globulin is also precipitated. Its amount is very small. Filter off the precipitate, and treat it with water. The caseinogenate of calcium dissolves, giving an opalescent solution. Heat the filtrate from the precipitate, the lactalbumin is coagulated. The lactalbumin can be precipated by saturating the solution with ammonium sulphate crystals. Note that the calcium caseinogenate behaves like a globulin.

2. Dilute 25 c.c. of milk with three times its volume of water. Then add dilute acetic acid, drop by drop, shaking after each addition of acid; a flocculent precipitate of caseinogen is formed, which mechanically carries down the fat with it. Add the acid until a precipitate is no longer formed and the solution appears quite clear. Carefully avoid excess of acid, which again dissolves the precipitate. Filter off the precipitate and wash it with water. *Keep the filtrate* for the separation of the other constituents.

Treat a portion of the precipitate with some dilute sodium carbonate solution. The caseinogen dissolves, leaving the fat in suspension. Filter through a wet filter paper and apply the colour reactions for proteins to the solution. All are positive. Also most of the precipitation reactions for proteins will be given.

Drain the remainder of the precipitate thoroughly and press it between filter paper. Transfer it to a dry test tube, and extract the fat with alcohol and then ether. The caseinogen can be completely freed from fat by continued extraction (p. 55).

Examine the pure caseinogen :—

1. The presence of the phosphorus has already been shown (p. 17).
2. It is insoluble in distilled water.
3. It has marked acid properties. Rub up some pure caseinogen with a solution of ammonium carbonate, or sodium carbonate, and a suspension of calcium carbonate in a mortar. In each case the caseinogen forms a soluble salt expelling carbon dioxide. Heat the solutions of these salts to 40° C. The calcium salt becomes milky in appearance; this disappears again on cooling.

To some of the sodium caseinogenate solution add a little calcium chloride solution; an opalescence is produced owing to the formation of the calcium salt.

Filtrate from the Separation of Caseinogen.

It has a distinctly acid reaction. Boil and whilst boiling nearly neutralize with dilute sodium carbonate solution. (If over neutralized, dilute acetic acid must be added till the reaction is faintly acid.) Coagulation of lactoglobulin and lactalbumin takes place. Filter off the precipitate and test it for protein.

The filtrate contains the lactose and calcium phosphate and other salts :—

1. Test with Fehling's solution. Reduction occurs.

2. Prepare lactosazone by treating with a few drops of phenylhydrazine and glacial acetic acid. Examine the crystals under the miscroscope to show that they consist of lactosazone.

3. Acidify strongly with nitric acid, add ammonium molybdate and warm; a yellow precipitate of ammonium phosphomolybdate forms. Or add magnesia mixture and shake; a precipitate of ammonium magnesium phosphate separates out. P. 17.

Separation of the Constituents of Cheese.

1. Rub up some grated cheese in a mortar with dilute sodium carbonate solution and filter. to dissolve caseinog

Acidify a portion of the filtrate. The casein is precipitated, but is soluble in excess of acid.

Apply the colour reactions and other reactions for proteins to the remainder of the filtrate.

2. Extract some grated cheese in a dry test tube with ether; pour off the ether into a basin and, after allowing the ether to evaporate, examine the residue for fat.

Why more distinct sulphur than in milk?
[illegible] less — Molisch's

D. EGGS.

Eggs consist of the shell, white, and yolk. The shell is made up of a membrane on which are deposited lime salts, chiefly calcium carbonate. The membrane is a keratin belonging to the group of scleroproteins.

The white contains, as previously seen, ovomucin or globulin and albumin, the former in small quantities, but the latter to about 10 per cent.

Another protein is still present—ovomucoid, a glucoprotein. It remains in solution after the coagulable proteins have been removed by boiling.

The yolk consists of fat, lecithin, lutein the yellow colouring matter, cholesterol and vitellin, a phosphoprotein.

The fat, some of the lecithin, lutein, and cholesterol can be extracted from the yolk with ether.

The residue, which becomes quite colourless, consists of lecitho-vitellin, and is soluble in 10 per cent. sodium chloride solution. The lecithin which it still contains can only be extracted by boiling with alcohol. The vitellin then remains, which is insoluble in salt solutions.

The Constituents of Egg-white.

Boil about 50 c.c. of the egg-white solution in a porcelain basin and whilst boiling faintly acidify. Filter off the coagulated proteins and examine the filtrate :—

[illegible]

1. It is not precipitated by mercuric chloride, but it is precipitated by tannic acid, and by phosphotungstic acid in acid solution.

2. It gives Millon's reaction. Examine also the other colour reactions for proteins.

3. Boil about 10 c.c. with an equal volume of concentrated hydrochloric acid for five minutes, cool, neutralize, and test for reducing sugar with Fehling's solution. The cuprous oxide is generally best seen on allowing to stand, when it settles to the bottom of the tube.

4. Evaporate the remainder on the water bath to dryness. A horny substance is obtained which, when treated with water, swells up and becomes gelatinous.

A similar glucoprotein—serum mucoid—is present in serum.

The Constituents of Egg-yolk.

Egg-yolks have been extracted with ether: the ethereal extract has been distilled to remove the ether and the residue extracted with alcohol, which dissolves the lecithin leaving a mixture of fat and protein. (This protein has dissolved in the ether in the presence of fats, lecithin, and water.)

I. The Alcoholic Solution of Lecithin.

Like fats, lecithin is not soluble in water, but it is soluble in alcohol and ether; unlike fats, it is insoluble in acetone. On adding acetone to a concentrated ethereal solution lecithin is precipitated.

1. It gives a white emulsion on pouring into water.

2. It gives a white precipitate of lecithin-cadmium chloride on adding a solution of cadmium chloride in alcohol.

3. Boil a small quantity, from which the alcohol has been evaporated, with caustic soda. Trimethylamine (from the choline) is evolved. Fatty acids are precipitated on acidifying. The filtrate contains choline and glycerophosphoric acid. P. 101.

II. The residue of Lecitho-Vitellin in 10 per cent. sodium chloride solution.

1. It coagulates at 70 to 75° C. on heating.

2. Pour about 50 c.c. of the solution into a large quantity of water, faintly acidified with dilute acetic acid. The lecitho-vitellin is precipitated. (Compare globulin: soluble in salt solutions, insoluble in water.) Allow the precipitate to settle, pour off the clear solution and filter the remainder. Treat the precipitate with boiling alcohol to remove the lecithin and examine the residue of vitellin:—

(*a*) Apply some of the colour reactions for proteins.

(*b*) The presence of the phosphorus in vitellin can be shown by boiling a portion of this precipitate for a short time with caustic soda, acidifying with nitric acid, filtering from any precipitate which may form and testing for phosphates with nitric acid and ammonium molybdate. Phosphoproteins may be distinguished in this way from nucleoproteins. P. 17.

(*c*) Observe that on digestion with pepsin and hydrochloric acid an insoluble residue, which contains a large percentage of phosphorus, remains. Nucleoproteins also leave a residue on digestion. Caseinogen leaves a similar residue, "paranuclein," but the greater part of its phosphorus goes into solution. This residue in all cases is nuclein. It arises from nucleoprotein, which is present in caseinogen and vitellin as an impurity.

E. **MEAT (Muscle).**

Muscle consists essentially of soluble proteins. The principal other constituents are fat, extractives (creatine) and connective tissue.

The soluble proteins present in living muscle are paramyosinogen and myosinogen in the proportions of one-fifth and four-fifths respectively. As the result of coagulation—*rigor mortis*—these are converted into myosin, the former directly, but the latter through the stage of soluble myosin, thus

$$\left.\begin{array}{l}\text{paramyosinogen} \qquad\qquad\qquad\quad \rightarrow \\ \text{myosinogen} \rightarrow \text{soluble myosin} \rightarrow\end{array}\right\}\text{myosin.}$$

This change is accelerated by acids and by a rise of temperature; it does not occur in weak alkaline solutions or in the absence of salts. Normally the change is brought about by lactic acid, and the disappearance of rigor is owing to the re-solution of the coagulated protein by the lactic acid. According to Mellanby ("J. Physiol.," 1908, Proc.) there is only one protein in living muscle.

There is still another constituent of our meat food, namely **Nucleoprotein,** which has not yet been mentioned. This is contained in greatest amount in organs such as the thymus, lymphatic glands, pancreas, etc. It consists of a combination of nucleic acid with protein: in the case of fish-roes the protein is a protamine or a histone; in that of the thymus the protein is histone and coagulable protein. The nucleic acids obtained from the different organs are also different, and are composed of purine bases—adenine, guanine, etc. These are supposed to give rise to the uric acid in the urine.

The Constituents of Muscle.

A. An aqueous extract of muscle has been prepared by extracting fresh veal with 0·9 per cent. sodium chloride solution in the presence of a little toluene to prevent putrefaction, straining through calico and squeezing out the solvent.

B. A saline extract has been prepared by grinding up the residue from A with sand and extracting it with five times its volume of 10 per cent. ammonium chloride solution and straining from connective tissue, sand, etc.

C. An extract of the lymphatic glands of the ox or sheep, or the thymus of a calf has been prepared by removing the fat, finely mincing, grinding up with sand and extracting for twelve hours with 10 to 12 times their weight of water containing a little toluene to prevent putrefaction. During this time it is well shaken and then the fluid is strained through muslin and centrifugalized. Owing to the nature of the nucleoprotein filtration is almost impossible.

A.

1. An aqueous extract of perfectly fresh muscle is alkaline, but it quickly becomes acid. This acid is lactic acid. Test the solution for lactic acid with Uffelmann's reagent (2 per cent. phenol solution treated with ferric chloride till of an amethyst violet colour): it changes to yellow.

2. Place a small quantity in the water bath at 40° C. for some time; a precipitate consisting of myosin from unchanged myosinogen may occur.

Filter off this precipitate and heat the filtrate further to about 70° C. A further coagulation of proteins occurs. This will consist of proteins from blood serum and any still unchanged myosinogen.

B.

Pour some of the solution into a large volume of water. The myosin is precipitated. Allow the precipitate to settle and then decant off the supernatant liquid. Examine the suspension of myosinogen which remains, as follows:—

1. To a small quantity add saturated sodium chloride solution, drop by drop. The precipitate dissolves. Now saturate the solution with sodium chloride crystals; the myosin is reprecipitated.

2. Add saturated ammonium sulphate solution, drop by drop, to another small portion. The myosin again dissolves. It is reprecipitated on adding an equal volume of saturated ammonium sulphate solution. Hence myosin is a globulin.

3. It coagulates at about 57° C. when dissolved in a little ammonium sulphate solution.

4. Prove that it is protein that is present by applying the colour tests for proteins.

C.

Add dilute acetic acid till no more is precipitated, and place in the water bath at 37° C. for 2 to 3 minutes to help the separation of the nucleoprotein and nucleohistone. Allow it to settle and pour off the liquid. Test as follows:—

1. The precipitate dissolves on adding alkali, and is thrown down again on acidifying. It is soluble in excess of acetic acid forming an opalescent solution. (Distinction from mucin.)
2. Show that it is a protein by the colour tests, etc.

The nucleohistone is precipitated by adding dilute calcium chloride to the extract and warming to 37° C. On decanting the liquid and acidifying it with acetic acid the nucleoprotein is thrown down.

3. The presence of phosphorus is detected by incinerating a portion of the precipitate with fusion mixture and testing the ash in the usual way for phosphoric acid.
4. Notice that on pepsin digestion it leaves a considerable residue, which contains the greater part of the phosphorus (nuclein), the protein having been dissolved by the pepsin.

F. CEREALS (Wheat, Barley, etc.).

The chief constituents are starch and protein. Fat and salts are also present. The protein consists of

(*a*) Gliadin, which is soluble in 70 to 80 per cent. alcohol.

(*b*) Glutelin, which is insoluble in alcohol and water, but is soluble in very dilute alkali and acid.

The same constituents exist in bread as in flour, but the starch has been partly fermented (1) by the diastase in the grain and converted into dextrin and maltose; (2) by the enzymes of the yeast which convert the dextrin and maltose into firstly glucose and secondly alcohol. The crumb will generally contain starch, dextrin, maltose, and glucose, but the crust will contain scarcely any glucose, as in the process of baking it is changed into caramel. Bread and cakes baked with baking powder contain as a rule no reducing carbohydrate, as this inhibits the action of the diastase; further, no glucose will be present as no yeast is used.

The Constituents of Wheat-Flour.

Knead some wheat flour with water to form a dough, and allow it to stand for a short time. Then wrap up a portion in muslin and knead it in a basin of water. Note the deposit of starch grains. Examine them under the microscope and compare with those of potato. Boil some with water, cool and test with iodine. Completely remove the starch by kneading in a stream of water, until no more grains pass through. A yellow gelatinous mass remains behind. This is termed gluten, and it contains the two proteins of wheat. Prove this by the protein colour tests.

Extract the gliadin from the gluten by boiling it out twice with alcohol. Filter. Evaporate off the alcohol from the filtrate and note the residue. It will give the protein tests.

The remainder is glutelin. Observe that it is:—

Sol-like metaprotein

1. Insoluble in water. not albumen
2. Soluble in dilute acetic acid. ~~not a metaprotein~~
3. Soluble in dilute caustic soda (0·4 per cent.).
4. Insoluble in salt solutions. not globulin

The Carbohydrates in Bread.

Extract some crumb and crust of bread with water and test the filtered extracts for carbohydrates (starch, dextrin, glucose).

G. VEGETABLES.

Potatoes consist chiefly of the carbohydrates, cellulose and starch. Protein is only present in small quantities, and fats in traces.

The principal constituents of the legumes (peas, beans) are protein and starch; fats are only present in very small amount.

The pea, horse bean, lentil and vetch contain as principal protein a globulin, known as legumin; another globulin—vicilin—is present in the three first mentioned. These seeds also contain small quantities of an albumin, termed legumelin, which resembles the albumin, leucosin, contained in wheat. The legumin and vicilin are separated from one another by fractional precipitation with ammonium sulphate.

The green leaves of vegetables are composed mainly of water and the solid constituents consist of cellulose, chlorophyll. The amount of nutriment in them is negligible.

1. The Constituents of Potato.

Scrape the clean surface of a piece of potato and collect the scrapings in a beaker. Add a little sodium chloride, stir well and strain through calico into another beaker. Note the deposit of starch on standing. This can be obtained pure by stirring with water two or three times and allowing to settle. Examine under the microscope and compare with the starch grains from wheat.

Test the supernatant liquid for proteins.

The Constituents of Peas.

Extract about 10 gm. of the pea flour with 10 per cent. sodium chloride solution. Filter. Test the residue for starch. Saturate the clear filtrate with ammonium sulphate crystals (80 gm. per 100 c.c.). The legumin and vicilin are precipitated. Filter. Redissolve the precipitate in dilute ammonium sulphate solution ($\frac{1}{100}$ saturated) and precipitate the legumin by adding saturated ammonium sulphate in the proportion of 150 c.c. to 100 c.c. solution ($\frac{6}{10}$ saturation). On dissolving the legumin in 10 per cent. sodium chloride solution and boiling no coagulum is formed.

The vicilin is obtained by nearly saturating the filtrate from the legumin with ammonium sulphate. It is coagulated on boiling its solution in 10 per cent. sodium chloride.

As globulins, these proteins are precipitated by pouring their solutions in salts into a large volume of water.

H. FRUITS.

Fruits consist principally of water and cellulose. They contain scarcely any foodstuffs. In bananas there is a considerable amount of starch. Malic, tartaric, and citric acids are the acid constituents.

Nuts consist mainly of protein (globulins) and fat (oil).

Excelsin, amandin, juglansin are respectively the globulins contained in Brazil nuts, almonds, and walnuts. They are isolated by extracting the ground-up nut with salt solution of 10 per cent. after previous removal of the oil by means of petroleum ether.

The Constituents of Nuts.

1. Extract a small quantity, about 2 gm., of the ground-up nut with ether. Filter. Allow the ether to evaporate and note the residue of oil.

2. Extract about 10 gm. of the fat-free nut meal twice with 50 c.c. of 10 per cent. sodium chloride solution. Pour the saline extract into about 20 volumes of water; a cloudiness is produced, which, on standing, gradually separates into flakes and settles to the bottom of the vessel. When the flakes have settled pour off the supernatant water and filter the remainder. Prove that the precipitate consists of protein.

(*Note.*—The protein of Brazil nut can be obtained in a beautifully crystalline state consisting of hexagonal plates if the saline extract be dialyzed: the separation of the globulin takes place more slowly in this way than by pouring into water.)

LOCALIZATION OF THE CHEMICAL CHANGES IN THE ORGANISM.

In the unicellular plants and animals the various chemical changes in the organic compounds must occur in the single cell. The colloidal substances are ingested and broken down by enzymes into crystalline and diffusible substances. These crystalline substances diffuse into the cell and are built up into colloids which again are broken down and converted into other crystalline substances capable of diffusing out of the cell. In the process of evolution of the higher plants and animals a differentiation of groups of cells into special tissues or organs has occurred. Like the single cell the cells of each organ must originally have been able to effect all the chemical changes, but during the evolution the power of effecting some of the changes has become lost, whilst the power of effecting other changes has become increased. Individual organs can effect most changes, but not all; some of the changes are effected by only one organ. Thus, for example, in animals, the organs of the alimentary canal are mainly concerned in the hydrolysis of the proteins, carbohydrates, and fats; the liver is concerned mainly in the metabolism of fats and carbohydrates, the conversion of ammonia into urea and the destruction and removal of blood pigment; the kidney is a filter which removes waste products; the suprarenal bodies, the thyroid and the spleen produce special substances to maintain the general equilibrium of the organism.

In plants, the differentiation is much less than in animals: the growing points and the cambium layer in the stems are the active tissues, also the cells in the flower or tuber which produce the seed and embryo.

THE CHANGES IN THE ALIMENTARY CANAL.

DIGESTION.

The three classes of compounds, the fats, the carbohydrates, and the proteins, after being taken in as food are hydrolyzed into their constituents before they can pass through the wall of the alimentary canal and be assimilated. These changes are produced by the soluble ferments or enzymes which are secreted by certain glands.

The enzyme (diastase or amylase) which acts upon starch and glycogen is produced by the salivary glands and the pancreas. Cane sugar is acted upon by invertase produced by the mucous membrane of the intestine. Lactose is hydrolyzed by lactase, present in the cells of the intestinal mucous membrane. Maltose is converted into glucose by maltase, which is present in the blood and the pancreatic juice. The natural β-glucosides, which are often taken medicinally, are also hydrolyzed into their constituents, probably by an enzyme (? emulsin) in the intestinal mucous membrane. The monosaccharides, so produced, are then decomposed into various products—carbon dioxide, lactic acid, alcohol (?), by enzymes in the various organs.

The fats are hydrolyzed by the enzyme lipase, which is present in pancreatic juice, the liver and other organs.

The proteins are hydrolyzed by:—(1) pepsin which is formed by the stomach, (2) trypsin which is derived from the pancreas, (3) erepsin in the small intestine, (4) proteoclastic enzymes in the various organs. The amino-acids produced are mostly deaminized by ferments in the various organs. The ammonia so formed is taken to the liver, converted into urea and excreted as urea by the kidney.

I. SALIVA.

The first digestion of food occurs in the mouth by the action of the saliva, which is the secretion of the salivary glands. This secretion contains mucin, the principal protein, ptyalin or salivary diastase, and generally also thiocyanic acid.

The action of the diastase of saliva is upon starch (and glycogen), which it converts into soluble starch, erythrodextrin, achroodextrin, and maltose (see p. 88). In the mouth, however, very little action takes place, the food being only moistened by the saliva and then swallowed. The action occurs in the stomach during the first half-hour, where the food is in the form of a mass in the fundus; here only the exterior of the mass is in contact with the hydrochloric acid of the gastric juice, which inhibits the action of the diastase whilst the interior of the mass is slowly digested.

A. The Constituents of Saliva.

1. Collect about 5 to 10 c.c. of saliva in a beaker. It has an alkaline reaction to litmus. It may be neutral or slightly acid at first, probably due to bacterial decomposition in the mouth.

2. Add acetic acid. Mucin is precipitated and is insoluble in excess of acid. Stir well with a glass rod; the mucin collects together and may be removed with the rod. The clear liquid which remains contains only traces of protein as exemplified, e.g. by Millon's reagent. The chief protein is therefore mucin.

3. Prove that the mucin is a protein by applying the xanthroproteic, Adamkiewicz, Millon's reactions, etc., to the above precipitate.

4. Mucin is soluble in 0·1 per cent. hydrochloric acid and 2 per cent. sodium carbonate solutions. It is a glucoprotein; on hydrolysis with acids it gives rise to a carbohydrate which reduces Fehling's solution. 2 glycopro

The mucin can be obtained from the submaxillary gland by grinding it up with sand and extracting it with 20 times its weight of 0·1 per cent. caustic soda. After about half an hour the fluid is strained through calico. The mucin it contains has the same properties as the mucin in the saliva.

5. Show the presence of thiocyanic acid by treating a little saliva with a drop of ferric chloride solution. The red colour is discharged by a drop of mercuric chloride solution. (Smokers' saliva generally contains more than that of non-smokers.)

6. The action of diastase on starch has already been examined (p. 88).

Comparison of the Digestive Activities of two Diastase Solutions.

In order to determine the activity of an enzyme solution four factors must be taken into account as was first clearly established by Kjeldahl in 1879 in the case of the diastatic enzyme of malt, namely:—

1. The temperature at which the action takes place.
2. The time during which the enzyme acts.
3. The amount of enzyme solution.
4. The concentration of the substrate solution.

By fixing any three of these factors the fourth may be determined. Thus, it will require *x* c.c. of an enzyme solution to completely convert 5 c.c. of 1 per cent. starch solution into maltose in 5 minutes, or it will take *x* minutes for 1 c.c. of enzyme solution to convert 5 c.c. of 1 per cent. starch solution into maltose. As basis of comparison it is best to determine the time taken to effect an equal change, rather than the amount of change in equal times. This is very important where the reaction takes place in stages; comparable values can be obtained only in this way.

In the case of diastase it is most usual to fix the temperature, the concentration of the substrate, and the time, and to estimate the amount of enzyme solution necessary to produce the change.

In practice either the maltose produced may be estimated by reduction of Fehling's solution or the absence of starch by the iodine reaction. It is more accurate to estimate the maltose, but it is simpler and more rapid to ascertain when starch is absent.

Omit

1. Lintner's Method.

In a series of ten clean test tubes is placed the same quantity of soluble starch solution (10 c.c. of 2 per cent.) and then a progressively increasing quantity of enzyme solution, thus 0·1 c.c. in No. 1, 0·2 c.c. in No. 2, 0·3 c.c. in No. 3, 0·4 c.c. in No. 4, etc. The contents are mixed and placed in a water bath at 21° C. for exactly 1 hour. Then into each tube is placed 5 c.c. of Fehling solution and the tubes are heated in a boiling water bath for 20 minutes after which time they are examined. One of the tubes in the series will show no blue colour, whilst the next on one side is faintly blue and on the other side colourless or faintly yellow. The amount of enzyme in this tube is that amount which will convert the fixed amount of starch into maltose in the given time.

The diastatic power is based upon 0·1 c.c. enzyme solution and called 100.

∴ if the result was between the sixth and seventh tubes the diastatic power

$$\text{is } \frac{100}{0{\cdot}65} \times 0{\cdot}1 = 15{\cdot}55.$$

2. Wohlgemuth's Method.

Wohlgemuth places 5 c.c. of 1 per cent. starch solution in a series of tubes and cools to 0° C. whilst an increasing quantity of enzyme solution is added to each tube in the series. He then transfers them to a bath at 40° C. for 30 or 60 minutes, and after this time again cools to 0° C. to stop the action. Each tube is then filled with water and one drop of $\frac{N}{10}$ iodine solution is added to each. The colours are blue, blue-violet, reddish, yellow. The limit is taken as that tube which still shows a violet colour. The activity of the solution is then calculated on the basis of the power of 1 c.c. enzyme solution. Thus if 0·3 c.c. enzyme solution were the tube in the series, then the diastatic power at 40° in 30 minutes or $D^{40°}_{30}$ is $\frac{5}{0{\cdot}3} = 16{\cdot}6$.

3. Roberts' Method.

Though not so accurate, this method is the most rapid to carry out. Here the time taken to effect the change of 1 per cent. starch solution into achroodextrin is measured. The first point when no colour is given by iodine solution, i.e. when the last traces of erythrodextrin have been converted into achroodextrin, is known as the achromic point. The time taken to reach this point is termed the "chromic period". The time taken to reach the achromic point must be between 2 and 10 minutes. The diastatic power D is the number of c.c. of starch solution which can be converted by 1 c.c. of enzyme solution in 5 minutes, or

$$D = \frac{n}{v} \times \frac{5}{t}$$

where n = number of c.c. of starch solution taken, v = volume of enzyme solution (dilution must be known), t = time, 5 = 5 minutes.

Compare the strengths of three saliva solutions prepared from saliva, (A) by diluting with 2 volumes of water, (B) by diluting with 4 volumes of water, (C) by diluting with 6 volumes of water as follows:—

Take 3 test tubes and put into them 5 c.c. of 1 per cent. starch solution and warm at 40° C. Then into test tube No. 1 put 5 c.c. of saliva solution (A), into No. 2 put 5 c.c. of saliva solution (B), and into No. 3 put 5 c.c. of saliva solution (C), keeping the tubes in the water bath at 40° C. Take the time at which the mixtures are made, and at intervals of ½ to 1 minute test the mixtures with iodine solution. Note when the achromic point is reached. The times taken will be as 1 : 2 : ~~4~~ 3

II. GASTRIC JUICE.

This is the secretion of certain cells of the mucous membrance of the stomach. Its chief constituents are hydrochloric acid and the enzymes pepsin and rennin. In disease lactic acid is sometimes found in the contents of the stomach besides the above. The stomach also contains the food and the products of the action of the enzymes on it.

Pepsin acts upon proteins in acid solution and converts them into metaprotein, proteoses and peptone, as has already been seen.

Besides pepsin, another enzyme, the rennet-ferment or rennin, is present in the mucous membrane of the stomach. This enzyme has the property of causing milk to clot, and so far as is known its action is limited to milk. The change which it produces in the milk consists in the conversion of the soluble calcium salt of caseinogen into the insoluble calcium salt of casein (or paracasein); in the process the caseinogen molecule is supposed by some to undergo hydrolysis, by others a rearrangement of the molecule is supposed to occur.

It has recently been put forward by Pawlow, Pekelharing and others that rennin is identical with pepsin; this is denied by Hammarsten, who was the first to distinguish their actions. If the action of rennin be identical with that of pepsin, the clotting of milk will then be the action of pepsin in neutral solutions upon milk. The identity of the two enzymes is emphasized by the fact that the proteoclastic enzymes of plants, such as are present in pine apple juice, etc., are also able to clot milk if their solutions be neutralized.

Rennin is obtained by extracting either the fourth stomach of the calf or the stomach of the pig with glycerol, and is used in making junket and cheese.

The Acids in the Gastric Contents.

The presence of hydrochloric acid and lactic acid in the gastric contents is determined by means of the following indicators :—

HCl

(*a*) Methyl-violet.
(*b*) Methyl-orange (dimethylaminoazobenzene). most delicate for [illegible]
(*c*) Congo red.
(*d*) Uffelmann's reageant.—2 per cent. phenol solution treated with very dilute ferric chloride till of an amethyst-violet colour. (Especially for lactic acid.)
(*e*) Gunzberg's reagent.—2 gm. phloroglucinol, 1 gm. vanillin, 30 gm. absolute alcohol (especially for hydrochloric acid).

The tests (*a*), (*b*), (*c*), (*d*), are carried out as follows :—

In a series of six clean test tubes place :—

1. 2 c.c. of 0·4 per cent. hydrochloric acid (the strength occurring in gastric juice).

2. 2 c.c. of dilute lactic acid (8 c.c. in 1000 c.c. water).

3. 2 c.c. of 0·4 per cent. hydrochloric acid and 2 c.c. of dilute lactic acid.

4. 2 c.c. of 0·4 per cent. hydrochloric acid and 2 c.c. of 2 per cent. Witte peptone solution in 5 per cent. sodium chloride solution. (This is to simulate the products of gastric digestion.)

5. 2 c.c. of dilute lactic acid and 2 c.c. of 2 per cent. Witte peptone solution in 5 per cent. sodium chloride solution.

6. 2 c.c. of 0·4 per cent. hydrochloric acid, 2 c.c. of dilute lactic acid, and 2 c.c. of Witte peptone solution.

Into each put 1 to 2 drops of methyl-violet solution. Note the change of colour and place the results in the table.

Then wash out the tubes and repeat, using methyl-orange solution, congo red, and Uffelmann's reagent.

The test with Gunzberg's reagent is carried out thus :—

Place in a small basin about ten drops of the solution and add 2 to 3 drops of freshly prepared Gunzberg's reagent. Evaporate very carefully over small flame, oscillating the basin and blowing upon the mixture. Charring must be prevented.

	Methyl-violet.	Methyl-orange.	Congo red.	Uffelmann's reagent.	Gunzberg's reagent.
HCl - - - - -	blue	pink	blue		purple
Lactic acid - - - -	red-violet	less " (1)	violet	yellow	no colour
HCl + lactic acid - -	[illegible]	bet (1)+(2)	bet (1)+2		pink
HCl + peptone - - -	yellowish	less p (1)	yellowish		no colour
Lactic acid + peptone - -	"	" " (2)	"	less yellow (2)	"
HCl + lactic acid + peptone-	"	bet 4+5	"		"

The Action of Rennin on Milk.

1. To 5 c.c. of milk add 2 c.c. of rennet solution.
2. To 5 c.c. of milk add 2 c.c. of boiled rennet solution.
3. To 5 c.c. of milk add 2 c.c. of sodium carbonate solution and 2 c.c. of rennet.
4. To 5 c.c. of milk add 1 c.c. of potassium oxalate solution and 2 c.c. of rennet.
5. To 5 c.c. of milk add 1 c.c. of potassium oxalate solution, 1 c.c. of calcium chloride solution and 2 c.c. of rennet solution.
6. To 5 c.c. of milk add 1 c.c. of potassium oxalate solution, 1 c.c. of calcium chloride solution and 2 c.c. of boiled rennet solution.

Place the six test tubes in the water bath at 40° C. for ten minutes and observe what has happened.

1. A clot has formed, and so firm that the tube can be inverted. On standing it contracts and a clear fluid—whey—exudes. The clot consists of casein and fat, and when pressed constitutes cheese. The whey contains the other constituents of milk.
2. No clot, as the ferment has been destroyed by heating.
3. No clot. The action of the ferment has been destroyed by the alkali. The reaction must always be neutral. If acid, the caseinogen itself will be precipitated. The rennet solution, if acid, must therefore be neutralized before use.

$$\left.\begin{array}{l}\text{4. No clot.}\\ \\ \text{5. Clot.}\end{array}\right\}\ \text{The presence of calcium is necessary to produce the clot.}$$

To (4) add some calcium chloride solution; a flocculent precipitate of calcium caseate is formed.

6. No Clot. Compare (2).

The changes that have occurred in the above experiments on the clotting of milk are :—

$$\text{Ca caseinogenate} + \text{rennin} = \underbrace{\text{Ca caseate}}_{\text{insoluble.}} + (\text{globulin ?}).$$

$$\text{Na caseinogenate} + \text{rennin} = \underbrace{\text{Na caseate}}_{\text{soluble.}} + (\text{globulin ?}).$$

$$2\text{Na caseate} + \text{CaCl}_2 = \underbrace{\text{Ca caseate}}_{\text{precipitate.}} + 2\text{NaCl}.$$

Comparison of the Digestive Powers of Two Pepsin Solutions.

The numerous methods which have been employed for comparing the strengths of two proteoclastic enzymes may be divided into two main groups :—

I. Those in which the action of the enzyme is determined by observing the rate of solution of an insoluble substrate.

II. Those in which the rate of formation of the products of the action of the enzyme is observed.

Of these the former is simpler for pepsin, the latter for trypsin.

(*a*) Brücke's Method.

In this method the time taken to dissolve equal-sized threads of fibrin was noted. The more active enzyme produced solution in the shorter time.

(*b*) Mett's Method.

Numerous results upon digestion by enzymes, especially those in Pawlow's laboratory, have been obtained by this method. It consists in directly measuring the amount of protein digested in a given time, the protein being contained in narrow tubes open at both ends and known as Mett's tubes.

Mett's tubes consist of a small length of coagulated egg-white or serum in a narrow glass tube of 2 mm. bore, and are made by drawing up egg-white into the glass tubing (no air bubbles must be present in the egg-white) and placing it in nearly boiling water for 2 to 3 minutes. This tubing is then cut up into lengths of about 1 cm. The coagulated egg-albumin must form a continuous layer free from air spaces. The small piece of tube is placed in the enzyme solution and after a definite lapse of time, the tube is laid upon a mm. scale and the amount dissolved from each end measured. The mean of these two readings is taken.

Observe the experiments which have been carried out by this method. Five c.c. of 0·4 per cent. hydrochloric acid and a Mett's tube were placed in each of the two small conical flasks; in the first was then placed 5 c.c. of pepsin solution No. 1 and in the second 5 c.c. of pepsin solution No. 2. The flasks were stoppered and kept at 37° C. for 8 to 10 hours. They were then removed, and the amount of protein digested from each end of the Mett's tubes was measured. Suppose 2 mm. and 4 mm. respectively were digested. According to the Schütz law when the action is for a long period of time the square roots of the lengths digested represent the activities of the two enzymes more accurately than the direct ratio. The activities of the two enzymes are therefore as 1·4 : 2 rather than 1 : 2.

(*c*) **Grützner's Method.**

This method is the one which has been and is most frequently employed on account of the rapidity with which the results are obtained. Fibrin, stained with carmine, is the substrate used; equal quantities are added to the two enzyme solutions and according to the rate of digestion of the fibrin more or less of the dyestuff passes into solution and comparison is made by observing the depth of the colour.

Roaf has suggested the use of fibrin stained with congo red instead of carmine. This possesses the advantage that the stained fibrin can be used in both acid and alkaline media; carmine-stained fibrin can only be used in acid media as carmine is dissolved out of the fibrin by alkalies.

The fibrin is prepared in the following way: Fresh fibrin is minced and washed till free from blood. It is then placed for 24 hours in a 0·5 per cent. solution of congo red, in the proportions of 50 gm. of fibrin to 100 c.c. of congo red solution. The mass is then poured into a large volume of water heated to 80° C. to fix the dye and kept at this temperature for about 5 minutes. The fibrin is then placed in a cloth and washed in running water; the excess of water is squeezed out and the fibrin is preserved in a mixture of equal parts of glycerol and water, a little toluene being added as preservative.

Another modification consists in the use of stained cubes of coagulated egg-white.

In two test tubes place exactly equal quantities of 0·5 gm. of congo-red fibrin and also 5 c.c. of 0·4 per cent. hydrochloric acid. To one of the tubes add 5 c.c. of pepsin solution A; to the other add 5 c.c. of pepsin solution B. Place the tubes in a water bath at 40° C. for half an hour. After this time add to each tube sufficient solid anhydrous sodium carbonate to change the blue colour of the congo red to red. This also stops the action of the enzyme. Now add water from a burette to the deeper one till the tints of the two solutions are the same. Note the amount of water added. The strengths of the enzymes are to one another as the amount of dilution: thus if an equal volume of water be added the strengths are as 2 : 1.

(*d*) Fuld's Method.

By making use of edestin as substrate and its precipitability by salts from its solution in hydrochloric acid, Fuld has devised a very simple method for comparing two pepsin solutions. Varying amounts of the enzyme solution are added to definite volumes of the edestin solution in a series of tubes and after a prescribed lapse of time sodium chloride is added; it is noted in which tube a precipitate of edestin is no longer formed. Thus:—

Measure out with a pipette 5 c.c. of the 0·5 per cent. edestin solution in 0·4 per cent. hydrochloric acid in each of a series of five test tubes. Add to these tubes in order, 0·2 c.c., 0·4 c.c., 0·6 c.c., 0·8 c.c., 1·0 c.c. of the pepsin solution A from a burette (generally 2 drops = 0·1 c.c.). Perform the same operations with pepsin solution B.

Keep all the tubes at the ordinary temperature for half an hour, or longer, but the same time for each series, then add to each tube 1 c.c. of saturated sodium chloride solution. Note in which tube of each series a precipitate of edestin is no longer produced. Suppose in the first case No. 2 tube, in second case No. 4 tube, then 0·4 c·c. of pepsin A = 0·8 c.c. of pepsin B. The activities of the enzymes are therefore as 4 : 8 or 1 : 2.

(*e*) Hata's Method.

It was shown by Hata in 1909 that a suspension of coagulated egg-white was a very delicate substrate for comparing the activities of two pepsin solutions. Under the influence of the enzyme the cloudy solution becomes quite clear. This method also possesses the advantage that egg-white is obtainable everywhere and the suspension is readily prepared. The comparison is carried out by adding varying quantities of enzyme solution to the substrate and observing in which tube clarification first occurs after a given time.

Preparation of substrate. Egg-white is rubbed up in a basin until it is of uniform consistence. It is then slowly mixed and rubbed up with water until it has been diluted five times. The solution is strained through gauze and heated in a water bath at 60° C. for 20 minutes, after which it is once more strained through gauze. A homogeneous suspension is thus obtained. Before use it is diluted with 9 volumes of water.

Measure out into a series of 5 test tubes 5 c.c. of the above substrate, add to each 1 c.c. of 0·4 per cent. hydrochloric acid solution and then in order 0·2, 0·4, 0·6, 0·8, and 1 c.c. of pepsin solution A. Perform the same operations with pepsin solution B.

Place all the tubes in a water bath at 40° C. for 15 or 30 minutes.

Note in which tube in each series the smallest amount of enzyme first produces complete clarification.

Suppose in the first series it is No. 3 tube and in the second series No. 4 tube; then 0·6 c.c. of enzyme A = 0·8 c.c. of enzyme B. The activities of the two enzymes are therefore as 6 : 8 or 1 : 1·3.

III. PANCREATIC JUICE.

The acid contents of the stomach when they pass into the duodenum and come in contact with the mucous membrane induce the production of secretin. This passes into the blood and is carried to the pancreas where it excites a flow of pancreatic juice. The juice so formed, however, has very little action upon proteins, but it contains lipase and diastase which act upon the fats and starch respectively, causing their hydrolysis. As soon, however, as the pancreatic juice, which contains trypsinogen, comes into the intestine, it becomes activated and converted into the powerful proteoclastic enzyme, trypsin, by the action of the enzyme enterokinase, which is secreted by the cells of the mucous membrane of the duodenum. The trypsin acts upon the proteoses, etc., from the stomach and converts them almost entirely into amino-acids—the last decomposition products of proteins. A complex polypeptide (perhaps the same as antipeptone) is the other product and this resists the action of trypsin.

1. Preparation.

Pancreatic juice has been prepared by the action of secretin and mixed with an equal volume of 2 per cent. sodium fluoride solution to act as an antiseptic. Secretin was obtained from the mucous membrane of the intestine by pounding it up with sand and boiling with dilute hydrochloric acid. When boiling, the mixture was neutralized in order to precipitate coagulable proteins and then filtered. The filtrate contains secretin. Note that secretin is not a ferment, as it can be boiled, but belongs to the class of substances termed **hormones** by Professor Starling.

2. Activation by Enterokinase.

(*a*) In three small conical flasks a Mett's tube containing gelatin[1] and 5 c.c. of 0·5 per cent. sodium carbonate solution have been placed. Into the first was put 1 to 2 c.c. of pancreatic juice, into the second 1 to 2 c.c. of pancreatic juice and a drop of enterokinase solution (extract of cells of mucous membrane), into the third 1 to 2 c.c. of boiled pancreatic juice and a drop of enterokinase solution. This acts as a control. The flasks have been kept at the ordinary temperature for 8 to 10 hours. Observe that no digestion has occurred in No. 1 and No. 3 where the trypsinogen has not been activated, or has been destroyed by boiling, but only in No. 2 where trypsin has been formed.

---

[1] Hot gelatin solution of 10 to 20 per cent. strength stained with methylene blue or gentian violet is drawn up into the tube and allowed to set; it is then cut into pieces of the required length. These tubes can only be used at the ordinary temperature, since at 37° C. the gelatin melts.

(*b*) H. Bierry and V. Henri have shown that milk is a very sensitive substrate for observing the activation of pancreatic juice by enterokinase. The milk is centrifugalized and filtered from fatty particles by filtration through wet paper and is sterilized by heating. Thus:—

Take four clean test tubes.

In No. 1 place 5 c.c. of milk + 5 drops of pancreatic juice.

In No. 2 place 5 c.c. of milk + 5 drops of pancreatic juice + 2 drops of intestinal extract.

In No. 3 place 5 c.c. of milk + 5 drops of boiled pancreatic juice + 2 drops of intestinal extract.

In No. 4 place 5 c.c. of milk + 2 drops of intestinal extract.

Put the four tubes in a water bath at 40° C. for 10 to 15 minutes. No change will be found to have occurred to tubes No. 1, No. 3, and No. 4, whereas in No. 2 there is an immediate clarification of the milk, which becomes transparent after the lapse of the above time.

3. **Detection of Amylase.**

Test about 1 c.c. of pancreatic juice (or a glycerol extract of pancreas) for the enzyme diastase, "amylopsin," with 1 per cent. starch solution just as previously done with saliva.

3 (*a*). **Detection of Lipase.**

Test also 1 c.c. of pancreatic juice or better a freshly prepared glycerol extract of pancreas[1] for the enzyme lipase as follows:—

Place about 5 c.c. of neutral olive oil in a test tube, add 5 c.c. of glycerol extract of pancreas and a drop of phenolphthalein. From a burette containing very dilute caustic soda solution add a few drops of alkali—just sufficient to colour the solution red. Place it in a water bath at 37° C. and shake occasionally. Decolorization will occur, due to the formation of free fatty acid from the oil by the enzyme lipase. From time to time run in, drop by drop, the alkali solution so as to keep the solution just coloured. Note the time when the experiment is commenced and when no more alkali is required to reproduce the red colour, as also the amount of alkali used.

Perform the same experiment, adding in addition a little bile. The time is considerably shortened. Bile therefore helps in the hydrolysis of fats by lipase. A control experiment with boiled enzyme solution should be carried out at the same time.

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[1] This is best prepared from a fresh pancreas of a pig. The pancreas is freed from fat, weighed, finely minced and ground up with sand. It is then extracted for twenty-four hours with a mixture consisting of ninety parts of pure glycerol and ten parts of 1 per cent. sodium carbonate solution, 10 c.c. of this mixture being used for every gramme of pancreas. The fluid is strained through muslin, and is kept at 0° C. The lipase is destroyed as soon as the fluid becomes acid; this happens generally in about three days.

4. Comparison of the Digestive Activities of Two Trypsin Solutions.

(*a*) Roberts' Method.

The action of trypsin upon milk serves as one of the most convenient and simple methods for determining not only its action, but also for comparing two trypsin solutions. Roberts in 1881 discovered that when trypsin acted upon milk a striking change in its properties occurred at an early stage in its digestion, namely coagulation on heating due to the formation of metacasein. This property disappears again at the end of the digestion.

Dilute 30 c.c. of milk with 30 c.c. of water in a small flask and warm to 40° C. Then add 1 to 5 c.c. of the trypsin solution. At the end of every minute withdraw with a pipette a portion of 5 c.c. and boil it for a few seconds. Notice at what time a distinct curdling occurs. Before the onset of the reaction the appearance of the tube on boiling is as if it had been soiled: this is due to incipient coagulation and it develops into curdling.

Carry out the same experiment with the trypsin solution which has been diluted with (1) an equal volume of water, and (2) with two volumes of water, adding the same amount of the diluted trypsin to the milk as used before with the undiluted trypsin. Take the time when the coagulation occurs.

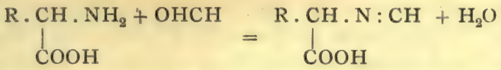
(*b*) Mett's Method.

The comparison of two or more trypsin solutions can also be made with Mett's tubes containing coagulated egg-white or gelatin. After periods of 2, 4, 8-24 hours the lengths digested from each end are measured. The ferments in a long period of time digest in the ratio of the square roots of the length digested.

Pure milk forms slight coagulation on boiling, hence dilution is absolutely necessary!

(*c*) Sörensen's Method.

The most accurate method, and simple also in the manner of carrying it out, is that of Sörensen. In this method the rate of formation of the products of the action of the enzyme, namely the amino acids, is measured. The amino acids contain both a carboxyl group and an amino group and consequently they react neutral. By combining the amino group with formaldehyde, its basic character is destroyed, and the carboxyl group is free to exert its acid character. The reaction which takes place is :—



$$\underset{\displaystyle\text{COOH}}{\text{R.CH.NH}_2} + \text{OHCH} = \underset{\displaystyle\text{COOH}}{\text{R.CH.N:CH}} + \text{H}_2\text{O}$$

Samples of a digest of caseinogen, gelatin, etc., with trypsin treated at intervals with neutral formaldehyde will show a gradual increase in acidity as the action of the enzyme proceeds and the rate of the increase will depend on the strength of the enzyme.

Neutralize 60 c.c. of formalin by running in $\frac{N}{10}$ alkali from a burette until its colour is just red to phenolphthalein, which is added as indicator.

Measure out 100 c.c. of a 4 per cent. caseinogen solution in 0·4 per cent. sodium carbonate solution into a small flask, warm it to 40° C. and then add, say, 5 c.c. of the trypsin solution ; keep the mixture at 40° C. Immediately after the addition remove with a pipette a sample of 25 c.c. and add 10 c.c. of the previously neutralized formaldehyde solution. At intervals of half an hour, one hour, one hour and an half, two hours, remove further samples of 25 c.c. and add to them 10 c.c. of the formaldehyde solution and a few drops of phenolphthalein. Titrate each sample as it is obtained with the $\frac{N}{10}$ alkali in the burette until the solution has a distinctly red colour. Note the amount of alkali used for each sample.

Carry out a similar series of operations with the trypsin solution B.

The ratios of the two enzyme solutions are to one another as the amounts of alkali added.

IV. INTESTINAL JUICE.

The intestinal juice, or succus entericus, is the product of the secretion of the cells of the mucous membrane of the small intestine. Not only has it an action upon proteins, but it also has the power of hydrolyzing the disaccharides. The proteolytic enzyme is peculiar in that it is only capable of acting upon the proteoses and peptone. It has been termed **erepsin** by Cohnheim.

Detection of Invertase.

1. An extract of the cells of the mucous membrane of the small intestine has been kept with some 1 per cent. cane sugar solution at 37° C. for about twelve hours. At the same time a boiled extract was kept to serve as a control. Test both extracts with Fehling's solution. Reduction only occurs with the first extract, which contains invertase.

Detection of Lactase.

2. The presence of lactase in the extract may be determined by the use of Barfoed's reaction as follows: A 1 per cent. solution of lactose has been kept for one day with an extract of mucous membrane in the presence of toluene, and at the same time a control with boiled extract has been kept under the same conditions.

Add 5 c.c. of each solution to 5 c.c. of Barfoed's reagent and immerse the two solutions in a boiling water bath for 3 to 5 minutes. Reduction will occur in the solution in which the unboiled enzyme was originally present. A slight reduction may occur in the other solution due to the presence of monosaccharide in the extract, but it will not be so marked as in the other solution.

Detection of Erepsin.

3. Examine the two solutions where some Witte peptone solution has been digested by an extract of mucous membrane and a control with boiled extract has been kept side by side with it. To each add the same quantity of caustic soda and the same number of drops of 1 per cent. copper sulphate solution. Compare the tints of the biuret reaction given. If the digestion has proceeded sufficiently long the biuret reaction will not be given in the solution where the enzyme has not been destroyed by boiling.

V. AUTOLYSIS.

A proteoclastic ferment is present in all tissues and is the cause of their autodigestion after death. The proteins are broken down into their constituent amino-acids. This occurs in starvation where one organ breaks down more rapidly than another, and hence is able to supply nourishment to those most in need of it. Not only is there a ferment capable of breaking down the simple proteins but also one capable of breaking down the conjugated protein, nucleo-protein, i.e. a nuclease, with the production of purine bases from the nuclein.

VI. PUTREFACTION.

This is the result of the action of bacteria, which are present in the large intestine. The same products, amino-acids, are formed, and in addition further products such as indole, scatole, hydrogen sulphide, mercaptan, which are the cause of the putrid odour.

VII. THE LIVER.

The liver is the organ which is concerned most intimately with the assimilation of the foodstuffs, especially carbohydrate and fat arriving from the alimentary canal, and with the regulation of their amount in the blood. Thus, glucose is converted into glycogen which is stored up as a reserve substance in the liver cells, to be broken down again when the amount of glucose in the blood sinks below its normal limit.

Glycogen, according to Külz, is contained in the greatest amount in the liver 14 to 16 hours after a meal. Its amount varies between 1 and 2 per cent. in well-nourished animals, but generally is much less, from 0·1 to 0·5 per cent. The glycogen content of the liver varies with the amount of muscular work. It has already been seen that glycogen is a colourless, tasteless powder which forms an opalescent solution in water and gives a reddish-brown coloration with iodine. It has a rotation of $[\alpha]_D = 196{\cdot}6^\circ$ and is precipitated from solution by alcohol (equal volume), tannic acid, or by saturation with magnesium or ammonium sulphate. It is hydrolyzed by acids to glucose, but it is not acted upon by strong caustic alkali. This serves as a method for its estimation.

The fats undergo their changes in the liver cells. The liver is probably also concerned in the building up of the proteins.

As a synthesizing agent the liver converts ammonia into urea (and uric acid in birds); and forms the ethereal sulphates and the glycuronates.

As a hydrolyzing agent the liver is concerned in the hydrolysis of arginine, which it converts into urea.

The rest of its function is excretory. The hæmoglobin in the blood is broken down in the liver, and the colouring matter, free from iron, is got rid of by the liver cells which secrete the bile.

The constituents of the bile are :—

1. The colouring matters, bilirubin and biliverdin, the latter formed by the oxidation of the former.

2. The bile salts, the sodium salts of glycocholic and taurocholic acid.

3. A small quantity of mucin or nucleoprotein (the more recent work insists on the presence of the latter, but both are probably present).

4. Cholesterol, which gives rise to gall-stones in certain conditions.

Estimation of Glycogen. (Pflüger.)

The liver of an animal is minced up and boiled for 2 to 3 hours on the water bath with 30 per cent. caustic potash. The alkaline solution is then diluted with water, filtered, and the glycogen precipitated with alcohol. After once more dissolving in dilute alkali and reprecipitating with alcohol, the glycogen is dissolved in a known volume of water and estimated by means of the polarimeter or by hydrolysis with acids into glucose—1 part glucose = 0·927 parts glycogen.

Examination of Ox or Sheep Bile.

1. It has a faintly alkaline reaction to litmus; note the bitter taste and peculiar odour.

2. It does not coagulate on heating.

3. Acidify a small quantity with acetic acid; a precipitate is formed which is insoluble in excess of acetic acid. As above stated, this precipitate used to be considered to be mucin owing to its insolubility in excess of acid, nucleoprotein being soluble. In the presence of bile salts the precipitate of nucleoprotein is insoluble.

P. 20

3*a*. Shake up a little bile with ether. No pigment is extracted. Now add a few drops of dilute hydrochloric acid; both nucleoprotein and pigment are liberated as free acids from their sodium compounds. The nucleoprotein is precipitated, but the pigment passes into solution in the ether.

4. **Gmelin's Test for Bile Pigments.** Carefully place a little bile on the surface of some fuming nitric acid in a test tube, either by pouring it carefully down the side of the tube or by means of a pipette. On shaking the tube very gently a play of colours will be seen as the bile becomes oxidized. Generally the colours are yellow, red, violet, blue, green;

or,

Place a drop of fuming nitric acid on a thin film of bile in a porcelain basin. Rings of the various colours will be seen;

or,

Filter a little bile several times through an ordinary filter paper and then place a drop of fuming nitric acid on the paper. Notice the colours.

5. **Huppert's Test for Bile Pigments.** This test is especially useful for detecting bile pigments in urine.

Dilute 5 c.c. of bile with 25 to 50 c.c. of water and add (1) 4 c.c. of sodium carbonate solution, (2) 6 c.c. of calcium chloride solution. Filter off the precipitate, which apparently carries down the pigment mechanically or may contain an insoluble calcium compound of bilirubin, and heat it with 5 c.c. of alcohol and a few drops of concentrated hydrochloric acid. A fine green colour is formed. The formation of the green colour may require the addition of an oxidizing agent such as a few drops of ferric chloride or potassium chlorate solution.

6. **Pettenkofer's Test for Bile Salts.** Dissolve a fragment of cane sugar in a little bile which has been diluted ten times with water; when it has dissolved run in about 5 c.c. of concentrated sulphuric acid to the bottom of the test tube and shake gently. A purple colour develops slowly. Furfural is formed by the action of the concentrated sulphuric acid on the sugar, and this reacts with the bile acids, giving the purple colour. Compare Molisch's reaction. Excess of sugar must be avoided as it may be charred by the strong acid and spoil the colour. The colour disappears on diluting with water, and is only stable in the presence of strong sulphuric acid. Dilute a portion of the purple liquid with 50 per cent. sulphuric acid and examine in the spectroscope. Two absorption bands, one between C and D nearer D, and the other in the green can be observed.

This test is sometimes performed by shaking up the bile with a little sugar solution so as to obtain a froth and then on pouring in the concentrated sulphuric acid the colour appears where it has come in contact with the froth.

7. **Hay's Test for Bile Salts.** (Surface Tension Test.) Dilute a little bile in a test tube with water. Sprinkle a little flowers of sulphur on the surface. Note that they sink. Repeat with pure water; the particles of sulphur float.

Perform the same test with strong mineral acids, ammonia, brine, ammonium sulphate solution, etc. The sulphur floats. It sinks in alcohol, ether, chloroform, olive oil, etc., in fact in all liquids with a surface tension less than 60 dynes per sq. cm.

This test depends upon the power of the bile salts to lower the surface tension of water. It is particularly valuable for detecting bile salts in urine where other coloured substances may interfere with Pettenkofer's test.

Alcohol, which has a low surface tension, must, if present, be previously removed by evaporation.

Grunbaum has described a method of estimating bile salts in urine which depends on this property of bile salts. The rate of escape of urine from standard capillary tubes is measured; the higher the concentration the greater is the rate.

Draughtsmen employ this property of bile salts when making tracings on oiled paper; the ink collects in drops and does not spread. On treating the paper with ox-bile and allowing it to dry the difficulty is overcome owing to the reduction in surface tension. Try this experiment with oiled paper treated with bile.

In the same way oil will pass through a filter paper moistened with dilute bile solution, whereas it will not pass through a paper moistened with water. Try this experiment.

8. **Solvent Action of Bile Salts on Fatty Acids Containing Oleic Acid.** Stir up some fatty acids from mutton or beef fat with water. None is dissolved. Add a little bile and stir up again. Fatty acids can be detected in the filtrate by evaporating to dryness, heating, and noting the characteristic odour. This process no doubt occurs in the digestion and absorption of fats.

Soap test

9. **Oliver's Test for Bile Salts.** This depends upon the power of the bile acids to precipitate peptone in acid solution, and is useful for showing the presence of bile salts in the urine.

Take about 20 c.c. of bile and evaporate it to complete dryness on the water bath. Heat the residue with 20 c.c. of alcohol on the water bath, stirring the mixture thoroughly with a glass rod. Add a little more alcohol. Filter. Evaporate the filtrate to dryness on the water bath, and then extract it with about 30 c.c. of hot water. Cool and filter. This gives a solution of the pigments and the salts of bile free from proteins.

Now acidify a portion of this solution with glacial acetic acid. The bile acids are not thrown down. On adding an equal quantity of 1 per cent. Witte peptone solution a turbidity or a precipitate is obtained, insoluble in excess of acid.

When applied to urine, it is only necessary to acidify with acetic acid, filter till quite clear, and treat with an equal volume of 1 per cent. Witte peptone solution.

10. **Cholesterol** may be detected as follows (Roaf):—

Evaporate 10 c.c. of bile to dryness on the water bath. Extract the residue several times with small quantities of ether, pouring each ether extract into another evaporating basin. Allow the ether to evaporate. Dissolve the residue in about 2 c.c. of chloroform and perform Salkowski's reaction (p. 139).

CIRCULATION OF CHEMICAL SUBSTANCES IN THE ORGANISM.

BLOOD.

The various chemical compounds, oxygen and carbon dioxide as carbonate, pass directly into or out of the lower organisms from the water or other medium in which they are living.

The higher organisms do not live in direct contact with water; and in their evolution a special medium has been differentiated for the circulation and transference of these substances from one organ to another. In plants, the medium is the cell sap, in animals the lymph and blood. In plants the oxygen and carbonates are simply dissolved in the cell sap. In animals the oxygen and carbonates are also dissolved in the lymph and blood, but the supply of oxygen in solution is not sufficient for the needs of the animals. Special cells, the red blood corpuscles, are elaborated by the bone marrow and spleen and passed into the blood. These cells contain hæmoglobin which readily combines with oxygen and as readily gives it up again. Blood also contains white blood corpuscles which are produced by the lymphatic glands and bone-marrow. These are living elements concerned in the removal of poisonous substances, etc., which may be introduced into the body by bacteria and by other agents.

Blood therefore is a fluid which contains the products of metabolism of the various organs and red and white corpuscles in suspension. The presence of blood platelets in living blood, i.e. in the blood vessels, is denied by some observers, but they are undoubtedly present when the blood is shed, their formation being instantaneous.

COAGULATION OF BLOOD.

Blood when drawn from a blood vessel, coagulates spontaneously into a solid mass or clot. On standing the clot slowly contracts, expressing the almost colourless blood-serum. The clot consists of the insoluble protein fibrin, which has entangled the blood corpuscles. The fibrin itself, almost free from corpuscles, can be obtained by whipping the blood whilst it coagulates, and then washing the fibrin threads with water to remove the entangled corpuscles; defibrinated blood remains, which contains the corpuscles. Thus:—

plasma
pl - corpuscles

$$\text{Blood}\begin{cases}\text{Fibrin + corpuscles.}\\ \\ \text{Serum.}\end{cases}\qquad\qquad \begin{matrix}\text{Blood}\\ \text{when}\\ \text{whipped}\end{matrix}\begin{cases}\text{Defibrinated blood}\\ \text{(serum + corpuscles).}\\ \text{Fibrin.}\end{cases}$$

We see then that in the process of clotting the insoluble protein, fibrin, is formed. It arises from fibrinogen, a soluble protein contained in the plasma, by the action of fibrin ferment or thrombin. Thrombin again does not exist in the blood, but is formed from precursors in the presence of calcium salts. According to Morawitz the precursors are thrombogen, which exists in the plasma as such, and thrombokinase, which is produced by the corpuscles or platelets, or from other tissues. Thrombokinase does not exist in bird's blood, which does not clot if carefully collected, but clots on adding a scraping of tissue, such as muscle. These substances were described under other names by Wooldridge, e.g. thrombogen = B fibrinogen, thrombokinase = A fibrinogen, fibrinogen = C fibrinogen.

According to the older observers, Alex. Schmidt, Hammarsten, Arthus, and quite recently Rettger, the corpuscles give rise to prothrombin, which is changed by calcium salts into thrombin. Thrombin according to Rettger, if obtained in solution almost free from protein, is stable to heat, i.e. it is not an enzyme.

The following scheme represents the processes which occur:—

$$\left.\begin{array}{l}\left.\begin{array}{l}\begin{array}{c}\text{Formerly}\\ \text{considered to be}\\ \text{Prothrombin.}\end{array}\left\{\begin{array}{l}\text{Thrombogen.}\\ \\ \text{Thrombokinase.}\end{array}\right.\\ \\ \qquad\qquad\qquad\text{Calcium salts.}\end{array}\right\}\text{Thrombin.}\\ \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad\text{Fibrinogen.}\end{array}\right\}\text{Fibrin + (soluble globulin ?)}$$

For their elucidation it was necessary to prevent the blood from coagulating, to prepare fibrinogen in a pure state from non-coagulated blood, to prepare a solution of fibrin ferment or thrombin, and to determine the factors which lead to the formation of thrombin.

Blood may be prevented from coagulating by collecting it in various salt solutions when it is drawn, e.g. sodium sulphate, magnesium sulphate, potassium oxalate, sodium fluoride, sodium citrate. Salt plasmas are thus obtained.

Coagulation may also be hindered or prevented by keeping the blood at a low temperature (0°), by injecting peptone into the circulation, by adding leech extract to blood or by injecting leech extract into the circulation. The blood when drawn does not coagulate. The active principle of leech extract can now be obtained under the name of hirudin.

Fibrinogen was first separated from salt plasma by Hammarsten by half saturating the plasma with sodium chloride, filtering off the precipitate, and washing it with half-saturated sodium chloride solution. When redissolved in water it coagulated at 56° C.

Fibrinogen can also be precipitated by ammonium sulphate, but less than half saturation with the salt is necessary, otherwise the serum globulin is also thrown down. It is obtained by diluting 12 c.c. of oxalate plasma with 30 c.c. of water and then adding 20 c.c. of saturated ammonium sulphate solution = $\frac{1}{4}$ saturation.

Fibrin ferment is not present in blood plasma, or present only in minute amounts. It is formed after the blood is shed from platelets or corpuscles (and thrombogen) in the presence of calcium salts. It will therefore be present in serum and on the fibrin, and may readily be prepared.

Formation of Serum and Separation of Fibrin.

Collect a little freshly drawn blood in two watch glasses. Allow the one to coagulate and observe that after a time it contracts, expressing the serum.

Defibrinate the blood in the second watch glass by stirring with a pin or needle, and notice that the fibrin adheres to the needle. The blood pigment can be washed away by water and almost colourless fibrin will remain.

Preparation of Fibrin Ferment.

(*a*) One volume of serum or defibrinated blood is mixed with 15 to 20 volumes of alcohol and the mixture is allowed to stand for some weeks. The precipitate is then filtered off, dried in a desiccator, and the ferment extracted with water. (Schmidt.)

(*b*) Fibrin ferment may be very readily made by extracting fibrin with .8 per cent. sodium chloride solution. It is best to use fibrin obtained from blood diluted with ten times its volume of water and washed. This fibrin can be preserved for some time in weak alcohol and the ferment then extracted. (Gamgee.)

EXPERIMENTS ON THE COAGULATION OF SALT PLASMAS.

Preparation.

(*a*) 1 part of blood is collected in 1 part of saturated sodium sulphate solution. 500 c.c. to 500 c.c.

(*b*) 3 parts of blood are collected in 1 part of saturated magnesium sulphate solution. 750 c.c. to 250 c.c.

(*c*) 9 parts of blood are collected in 1 part of potassium oxalate solution (1 per cent.). 900 c.c. to 100 c.c.

(*d*) 9 parts of blood are collected in 1 part of sodium fluoride solution (3 per cent.). 900 c.c. to 100 c.c.

During the mixing each is well shaken. The plasma is then separated from the corpuscles by centrifugalizing. All these plasmas should be quite free from blood corpuscles, as the stromata of these may serve as the mother substance of the ferment. It is difficult, however, to obtain them quite free from hæmoglobin, which gives them a reddish colour.

Extract some fibrin with 8 per cent. sodium chloride solution and use this or some serum in the following coagulation experiments with the salt plasmas.

(*a*) Sodium Sulphate Plasma.

1. Dilute a small quantity with five times its volume of water and keep warm at 40° C. Clotting occurs.

2. Dilute a small quantity with five times its volume of water, add a drop or two of fibrin ferment solution or serum (contains fibrin ferment) and keep at 40° C. It clots more quickly than (1).

3. Saturate a portion with sodium chloride. A precipitate, the plasmine of Denis (= fibrinogen + serum globulin) is formed. Dissolve it in water and warm to 40° C. It clots. Squeeze out the clot (fibrin); the fluid contains a soluble protein, the soluble fibrin of Denis (= serum globulin).

4. Add an equal volume of saturated sodium chloride solution. A precipitate of fibrinogen is formed. Wash it with half-saturated sodium chloride solution by decantation and redissolve it in water. If well washed, it will not clot at 40° C., but it will do so on adding serum or fibrin ferment solution.

Conclusions. This plasma contains fibrinogen and fibrin ferment. These are prevented from interacting by the presence of neutral salts, hence on dilution the plasma clots.

(*b*) Magnesium Sulphate Plasma.

1. Dilute a small quantity with nine times its volume of water and warm to 40° C. There is no clot.

2. Dilute a small quantity with nine times its volume of water and add a little serum or fibrin ferment and warm to 40° C. It clots. On standing this clot contracts expressing serum.

3. To some magnesium sulphate plasma add an equal volume of saturated sodium chloride solution. Fibrinogen is precipitated. Filter and wash the precipitate with half-saturated sodium chloride solution. Dissolve it in water and

(*a*) Determine the heat coagulation point with a small quantity = 56° C. Continue heating to 60° C. and filter. To the filtrate add serum and warm to 40° C. No clotting occurs.

(*b*) The solution does not clot by itself at 40° C., but coagulates on adding serum or fibrin ferment.

Conclusions. Magnesium sulphate plasma contains fibrinogen but no ferment; the formation of the ferment has been prevented by the presence of magnesium sulphate.

(*c*) Oxalate Plasma.

1. Dilute as before and warm to 40° C. No clotting occurs.

2. Dilute, add a few drops of calcium chloride solution, and warm to 40° C. Coagulation occurs.

3. Dilute and add serum to which a little oxalate has been added. It clots. Prove by filtering and testing the filtrate with calcium chloride that excess of oxalate was present. This experiment proves that calcium-free fibrinogen and calcium-free ferment form a clot.

N.B.—If the oxalate plasma has been kept at 0° C. for a few days and well centrifugalized a precipitate (thrombokinase) can be removed. In this case the plasma will clot with ferment, but not with calcium chloride.

4. To oxalate plasma add an equal volume of saturated sodium chloride solution (*Ca-free*). Wash the precipitate of fibrinogen with the same saturated sodium chloride solution. Dissolve it in water. This solution will clot, if the plasma is fresh (i.e. containing thrombokinase), with calcium chloride or serum. After the solution has clotted, it still contains a soluble protein —globulin.

Conclusions. The oxalate has prevented coagulation by removing calcium salts, hence on their addition, the blood coagulates. The calcium salts are concerned in the formation of the fibrin ferment or thrombin. Serum contains thrombin, and therefore, when it is added to blood free from calcium, coagulation occurs.

(*d*) Fluoride Plasma.

1. Dilute a portion with twice its volume of water and warm. Note the result.

2. Add a few drops of dilute calcium chloride solution and warm. No clotting occurs.

3. Add a little fibrin ferment or serum and warm. A clot forms.

4. Add a scraping of muscle and warm. It clots rapidly.

Conclusions. Fluoride plasma, if properly centrifugalized, contains no thrombokinase and no thrombogen, which is carried down with the calcium fluoride; hence it does not clot when calcium salts are added. If improperly centrifugalized, thrombogen is still present and also corpuscles, which give thrombokinase on diluting.

everything in
lymph

THE SOLUBLE CONSTITUENTS OF BLOOD PLASMA.

As the circulating medium for the distribution of the products formed by the various organs the soluble constituents of blood plasma will consist of proteins, fats, carbohydrates and their decomposition products. Blood plasma consists mainly of water; the solid matter amounts to less than 10 per cent. The proportions of the constituents are given in the following table:—

Water	917·6
Solids	82·4
	1000·0

Protein	69·5
Fat	1·2
Extractives	4·0
Soluble salts	6·4
Insoluble salts	1·7
	84·8

Protein thus makes up 70 per cent. of the total solid matter.

A. THE PROTEINS.

Blood plasma contains the three proteins, fibrinogen, serum globulin, serum albumin and also a nucleoprotein in small quantities. With the exception of fibrinogen the same proteins are contained in the serum. Serum also contains the other constituents. It is alkaline in reaction to litmus and has a specific gravity of about 1030.

B. THE FAT.

The fat may be separated from serum by drying and extracting with ether (p. 55).

C. THE EXTRACTIVES.

The extractives will consist of glucose, ammonia, urea, salts, etc. The presence of amino acids has not yet been definitely ascertained. The various organs may therefore make use of the soluble proteins in building up their protein constituents.

The extractives can only be examined after completely removing all proteins from solution. The proteins are precipitated by heat coagulation in acid solution, or by means of heavy metals, or by alkaloidal reagents. In the latter cases, the excess of reagent added must be removed before the examination can be carried out.

THE PROTEINS OF BLOOD SERUM.

The presence of protein may be shown by the colour and the precipitation reactions for proteins. Serum gives all the reactions which are given by egg-white.

Dilute 10 c.c. of serum to 100 c.c. with distilled water and carry out the following reactions :—

A. Colour Reactions :—

1. Biuret reaction.
2. Xanthoproteic reaction.
3. Millon's reaction.
4. Sulphur reaction.
5. Adamkiewicz reaction.
6. Molisch's reaction.

B. Precipitation Reactions :—

1. Heat Coagulation.

(*a*) Boil 5 c.c. in a test tube. The solution becomes opalescent, but there is no definite coagulum. — coag ppte

(*b*) Boil 5 c.c. with 1 to 2 drops of dilute acetic acid. A coagulum is formed. Cool and add 2 to 3 drops of concentrated nitric acid. The precipitate does not dissolve. The addition of the nitric acid ensures that the precipitate, formed on boiling, does not consist of calcium or magnesium phosphate, which is soluble in dilute nitric acid, e.g :—

Treat a solution of calcium chloride with sodium phosphate and then with excess of sodium carbonate. Calcium phosphate is precipitated. Add acetic acid, drop by drop, till the precipitate just dissolves ; acid calcium phosphate is formed. Boil for half a minute. A white precipitate of calcium phosphate forms which is due to the alteration of the reaction as the carbon dioxide is evolved. This dissolves on adding a drop or two of dilute nitric acid.

(*c*) Boil 5 c.c. with a drop of glacial acetic acid. No coagulation occurs.

(*d*) Boil 5 c.c. with 2 to 3 drops of dilute sodium carbonate solution. There is no coagulation.

The globulins and albumins of blood serum are thus only coagulated by boiling when the solution is neutral or very faintly acid. If the solution be alkaline, as serum generally is, as in (*a*) the proteins are acted on by the alkali as the temperature rises and converted into alkali-albumin. If strongly acid as in (*c*) the proteins are converted into acid-albumin. These compounds (metaproteins) are not coagulated on heating their solutions.

Temperature of Heat Coagulation.

Add a drop of dilute acetic acid to 5 c.c. of dilute serum in a test tube, and heat slowly in a beaker of water. Coagulation occurs at 70 to 82° C., chiefly between 73 to 75° C.

55°C. – faint cloudiness
82°C. – coagulation

2. **Alcohol.**

3. **Strong Mineral Acids.**

4. **Solutions of Heavy Metals.** Dilute serum is precipitated by mercuric chloride, copper sulphate, ferric chloride, lead acetate.

5. **Alkaloidal Reagents in Acid Solution.**

(*a*) Hydroferrocyanic acid.
(*b*) Picric acid.
(*c*) Potassio-mercuric iodide.
(*d*) Trichloracetic acid.
(*e*) Tannic acid.
(*f*) Bromine water.
(*g*) Phosphotungstic acid.

Estimate the amount of protein in the dilute serum by Esbach's method.

Separation of the Globulin and Albumin.

(*a*) **Sodium Chloride.** On saturating serum with crystals of sodium chloride a white precipitate of serum globulin is formed. Filter. The filtrate still contains protein as shown by the coagulation of the solution on boiling after acidifying it with dilute acetic acid.

(*b*) **Magnesium Sulphate.** Saturate some serum with magnesium sulphate. Note the white precipitate of serum globulin. Filter it off through a dry paper, and wash it with a little saturated magnesium sulphate solution. Scrape the precipitate off the paper and dissolve it in water. Boil the solution with a drop of dilute acetic acid. The serum globulin is coagulated.

The filtrate from the serum globulin contains the serum albumin. Boil a portion with a drop of dilute acetic acid. Coagulation occurs.

A white precipitate of serum albumin is formed on adding a drop of glacial acetic acid to another portion.

The serum albumin is precipitated by grinding up the remainder with sodium sulphate.

(*c*) **Ammonium Sulphate.** To 10 c.c. of undiluted serum add an exactly equal volume of saturated ammonium sulphate solution, thus making the solution half-saturated. Mix thoroughly, filter off the precipitate of serum globulin through a dry paper. Completely saturate the filtrate with finely powdered crystals of ammonium sulphate. The serum albumin is precipitated; filter it off through a dry paper. Scrape off these precipitates of serum globulin and serum albumin and dissolve them separately in water. Boil the solutions with a drop of acetic acid. Both are coagulated.

Albumins may thus be removed from solution by complete saturation with ammonium sulphate, or by combined saturation with magnesium sulphate and sodium sulphate.

The Serum Globulin.

The older methods of Panum and Schmidt for obtaining serum-globulin (i.e. by diluting serum with 15 or 20 times its volume of water and acidifying or passing in carbon dioxide, filtering off the precipitate and then acidifying) are now almost obsolete, as is also the preparation by dialysis. The newer methods by the addition of salts are practically confined to the use of ammonium sulphate. Saturation with sodium chloride and magnesium sulphate throws down the globulin but incompletely. By means of ammonium sulphate several fractions can be obtained which can be further separated by dialysis, etc. Thus:—

Euglobulin is pptd. by	30—37 °/o satn.	by dialysis	insol. para euglobulin + sol. euglobulin.
Pseudoglobulin ,,	37—44 °/o ,,		insol. para pseudoglobulin + sol. pseudoglobulin.
Globulin ,,	44—50 °/o ,,		(Freund and Joachim.)

(Porges and Spiro.)

(1) To some dilute serum add half its volume of saturated ammonium sulphate, thus making the solution one-third saturated. Filter off the euglobulin.

(2) To the filtrate add one-third of its volume of saturated ammonium sulphate solution, making the solution half saturated. Filter off the pseudoglobulin and globulin.

(3) Completely saturate the filtrate with powdered ammonium sulphate crystals. The serum albumin is precipitated.

Removal of Globulins and Albumins from Solution by Heat Coagulation.

Dilute the serum (egg-white, etc.) with ten times its volume of water, add 1 to 2 drops of dilute calcium chloride solution and a drop or two of litmus. Boil in porcelain dish, and, whilst boiling, add cautiously dilute acetic acid till the reaction is faintly acid. Filter and test the solution for proteins. If the experiment is properly carried out, it will be free from proteins.

A certain amount of metaprotein is first formed on heating. This is precipitated by the acetic acid and coagulated on boiling. Avoid excess of acid and add it slowly. The calcium chloride aids in aggregating the protein during the boiling.

DEFIBRINATED BLOOD.

Defibrinated blood contains the same soluble constituents as serum and in addition the white and the red blood corpuscles in suspension. Like blood-plasma it is alkaline in reaction and has a specific gravity of 1050 to 1055; the average specific gravity of human blood varies from 1041 to 1067.

Its most important constituent is the hæmoglobin contained in the red blood corpuscles. Hæmoglobin can be liberated from the corpuscles by laking the blood—hæmolysis. The amount of oxygen contained in blood depends directly upon the amount of hæmoglobin—oxygen capacity. Blood contains a very active catalytic enzyme, or catalase, which is termed hæmase; also a peroxidase. The peroxidase reaction is also given by boiled blood and is due to the iron constituent of the hæmoglobin.

1. Reaction.

Place a drop of defibrinated blood upon a piece of neutral glazed litmus paper. In 10 to 30 seconds, wash off the blood with distilled water; a blue stain remains, showing that the blood is alkaline in reaction.

2. Specific Gravity.

This is determined by placing a drop of blood into each of a series of solutions of sodium sulphate which have a specific gravity of 1·035 to 1·070 (Roy and Lloyd Jones' method), or by placing a drop of blood in a mixture of chloroform and benzene or light petroleum (Hammerschlag's method). The average is 1055 to 1060.

The sodium sulphate solutions are placed in a series of glass thimbles, and the blood from the finger is drawn up into a capillary tube (made by drawing out a glass tube in a flame and bending it at right angles) by means of a caoutchouc cap. It is placed into one of these solutions and it is noted whether it rises or sinks. Finally it will be found to neither rise nor sink in one of the solutions. The specific gravity of this solution is its specific gravity. In Hammerschlag's method, a mixture is made and a drop of blood placed in it, and either benzene or chloroform added until the drop neither rises nor sinks. The specific gravity is then taken with specific gravity beads, or with a hydrometer.

Introduce carefully a drop of defibrinated blood with a pipette into the centre of each of a series of sodium sulphate solutions of known specific gravity, and notice in which one it neither rises nor sinks, but remains at the same level. The blood will spread itself out, but it can be easily seen with which solution its specific gravity corresponds.

3. Laking of the Blood. Hæmolysis.

Notice the colour of the defibrinated blood, and that it is opaque even in very thin layers. The blood pigment which is normally contained in the red blood corpuscles may be discharged from them in several ways:—

(*a*) By diluting with 2 to 3 times its volume of water, or better by adding a little blood to excess of water. Water passes into the corpuscles by osmosis, bursts them, and liberates the hæmoglobin. Observe that it has become transparent and has a slightly different tint. Show that this does not occur when blood is mixed with 0·9 per cent. sodium chloride solution, since the contents of the corpuscles and the saline solution have the same osmotic pressure.

(*b*) By adding a little weak alkali.

(*c*) By shaking up with a few drops of chloroform or ether.

(*d*) By placing in a freezing mixture of ice and salt, and then warming to 50° C.

To observe laking in (*b*) (*c*) (*d*) dilute subsequently with 0·9 per cent. sodium chloride solution, not with water which by itself lakes the blood.

4. The Catalytic Action.

To a little dilute defibrinated blood add a little hydrogen peroxide and notice the evolution of oxygen. This is also due to the blood pigment, but is more extensive with fresh blood where a catalase (i.e. an enzyme which acts on hydrogen peroxide giving molecular oxygen) is also present.

5. The Guaiacum Reaction.

Dilute considerably a little defibrinated blood (2 to 3 drops in a test tube full of water). Then add about one-eighth of its volume of hydrogen peroxide (10 vols. per cent.) and float on the surface a little tincture of guaiacum or a solution of guaiaconic acid (the essential constituent of guaiacum). A blue colour (guaiacum blue) will gradually develop above the resinous ring and slowly diffuse into the clear zone above this. The blue colour ultimately disappears.

This reaction succeeds with blood diluted 1 in 10,000, also with old blood stains, carboxy-hæmoglobin, hæmochromogen, hæmin crystals, etc. It is also given by boiled blood solutions. (*Buckmaster.*) It thus differs from the reaction with milk and other substances where an oxidase is necessary and the reaction does not succeed after boiling. It depends apparently on the iron constituent of the hæmatin.

Boil a dilute solution of blood, and test the clear filtrate with hydrogen peroxide and guaiaconic acid or tincture of guaiacum.

6. The Oxygen Capacity.

This can be readily determined by Haldane's ferricyanide method, which consists in estimating the amount of oxygen liberated when potassium ferricyanide is added to blood. It is more rapid and probably more accurate than the older method of removing the oxygen by means of the vacuum pump. The same apparatus can be used as is employed in the estimation of urea (p. 112).

Thoroughly saturate about 30 c.c. of defibrinated blood with air by spreading it over the surface of a large flask. With a pipette measure out 20 c.c. and introduce it into a bottle of about 120 c.c. capacity. The last drops of blood must not be blown out, but are expelled by closing the top of the pipette with the finger and warming the bulb with the hand. Then add to the blood 30 c.c. of dilute ammonia solution (1 part ammonia of sp. gr. ·880 in 500 parts distilled water). The ammonia prevents the evolution of carbonic acid whilst the water lakes the corpuscles. Mix the blood and ammonia. The solution should be quite transparent. If the laking is not complete, more ammonia must be added. Next put 4 c.c. of saturated potassium ferricyanide solution in a small tube, which should be slightly longer than the width of the bottle, and place it upright in the large bottle without spilling. Close the bottle with a rubber stopper through which a glass T tube passes and which is connected at one end with a burette in a cylinder of water by india-rubber tubing and closed at the other end with a clip. Put the bottle in a vessel of water of the same temperature as that of the room. Bring, by opening the clip, the level of the water in the burette nearly to the top and to the same height as the water in the cylinder. Close the clip and read off the height in the burette. Then tilt the bottle so as to upset the ferricyanide solution and shake gently as long as gas is evolved, repeatedly emptying the little tube so that all the oxygen is given off. When the oxygen ceases to be evolved, replace the bottle in the water, and add cold or hot water to it until it attains the same temperature as at the commencement. [This is done by means of a pressure gauge for very accurate work. See Haldane, "J. Physiol.," vol. 25.] Now adjust the burette so that the water has the same level inside and outside and read off the volume of gas (the burette should be graduated to 0·05 c.c.). The difference in readings gives the amount of oxygen evolved from 20 c.c. of blood.

This volume should be reduced to 0° and 760 mm. and a correction should be made for the pipette which delivers only about 19·6 c.c. blood instead of 20 c.c. blood. Further, in very accurate work account must be taken of the air in the bottle, which has become richer in oxygen and oxygen is twice as soluble as nitrogen.

About 4 c.c. of oxygen are given off by 20 c.c. of fresh ox blood.

7. The Estimation of Hæmoglobin.

The various methods which have been used depend upon the comparison of the colour of the blood with a series of standard colours, or with a solution of fixed colour by diluting the blood until it attains a similar tint. Oliver's, von Fleischl's, and Tallquist's methods depend upon the first process; Hoppe-Seyler's, Gowers', and Haldane's methods upon the second process.

1. *Tallquist's Method.*

Touch a drop of blood with a piece of filter paper and allow the blood to diffuse through the paper so as to give an even stain. Place this beside the colour scale which is graduated in percentages of hæmoglobin from 10 to 100, and move it till it matches in tint one of the colours. It may be deeper in colour than 90 but less than 100, and hence the percentage lies between 90 and 100. The method is not very accurate, but it gives good average results.

2. *Von Fleischl's Method.*

A quantity of blood (20 c.mm.) is diluted 100 times with water and placed in one compartment of a circular trough, whilst in the other compartment is placed the same quantity of distilled water. Under the trough in which the water is placed a wedge of coloured glass attached to a graduated scale is capable of being moved to and fro. It is moved until the colours match one another. The scale, which is graduated in percentages of hæmoglobin, is then read.

3. *Oliver's Method.*

The sample of blood of known volume is diluted in a small cell with water and its tint is compared with the colour of a series of coloured glasses which are tinted to match known strengths of hæmoglobin solutions.

4. *Hoppe-Seyler's Method.*

In this method two parallel-sided glass troughs are used; into one is placed standard blood, into the other a known volume of the blood to be tested: this is diluted with water until the tints are the same and the amount of dilution is noted. The standard blood is 1 per cent.; 5 c.c. of normal blood are saturated with carbon monoxide to convert the hæmoglobin into carboxyhæmoglobin which is more stable than hæmoglobin, and diluted with water to 500 c.c.

The method can be rapidly illustrated by using two test tubes of equal bore :—

Into one of the two test tubes place 10 c.c. of standard hæmoglobin solution. Into the other tube place 5 c.c. of water and drop into it $\frac{1}{10}$ c.c. of the blood whose hæmoglobin content is to be determined. Pass into it a little coal gas, taking care not to touch the liquid with the tube, and avoid spilling or frothing. Shake up carefully and repeat the process to ensure that all the hæmoglobin is converted into carboxyhæmoglobin (see p. 222).

Compare the colour with that of the standard tube by holding the tubes against white paper. Then from a burette run in water, drop by drop, until the colour matches that of the standard tube. It is best to note the number of c.c. run in when it appears of the same colour as the standard, and then to add water till it appears appreciably lighter. Take the mean of the two readings. From the quantity of water added the percentage of hæmoglobin in the undiluted blood can be calculated.

5. *Gowers' Method.*

Instead of using blood as the standard solution for comparison Gowers uses glycerin jelly stained with carmine. This has been prepared by comparison with normal blood diluted 100 times with water, and is contained in a small sealed tube. The comparison is made in a tube of the same capacity and graduated in percentages. Into this tube is placed 20 c.mm. of blood and it is diluted until its tint is the same as the standard. The method of estimation is carried out in the same way as in Haldane's method.

6. *Haldane's Method.*

Haldane has modified Gowers' method by using a standard solution of ox blood or sheep's blood saturated with carbon monoxide in the place of the carmine-tinted glycerin jelly. The oxygen capacity of the standard blood is 18·5 per cent. This corresponds to 13·8 per cent. of hæmoglobin. Since 1 gm. of hæmoglobin can combine with 1·34 c.c. oxygen, it is the same as that of adult males.

The apparatus necessary for the estimation is the standard tube (D), a companion tube graduated in percentages (C), a lancet for pricking the finger (F), a pipette to measure 20 c.mm. of blood (B), a tube to pass carbon monoxide or coal gas into the blood, and a bottle of distilled water with a drop pipette for gradually diluting the blood (A). The items are shown in Fig. 39.

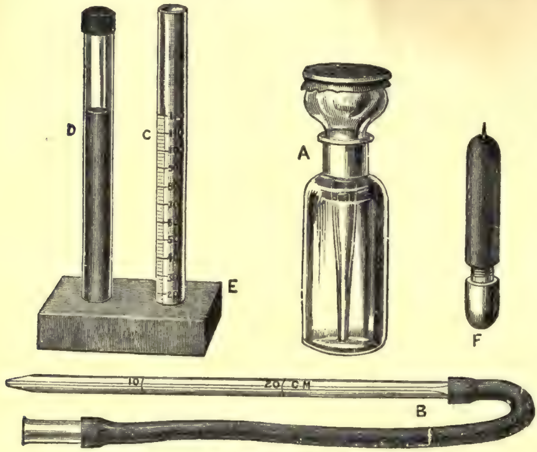


FIG. 39.

The process of estimation is carried out as follows:—

Place in the small tube, which is graduated in percentages from 0 to 100 or 120, less water than will be ultimately needed to dilute the blood to the required tint. It contains 2 c.c. when filled up to the mark 100.

Prick the finger with the small lancet and fill the capillary pipette to a little beyond the mark 20 (= 20 c.mm. or 0·02 c.c.) by sucking up the blood into it. Wipe the point of the capillary pipette and dab it on filter paper till the blood stands exactly on the mark. Then blow the blood into the water in the measuring tube. Attach the cap of the special tube for the purpose to a gas burner and pass coal gas into the tube, removing the cap before turning off the gas. Immediately close the tube with the finger and move it so that the liquid passes from end to end at least a dozen times. The tube should be held in a cloth to prevent liquid spurting out when the finger is removed owing to the tube becoming heated. Now add distilled water, drop by drop, with the pipette stopper of the bottle, the tube being inverted after each addition till the tints are the same in both tubes. It is best to take two readings (1) when the colours are the same, (2) when the colour is appreciably lighter and then to take the mean. The percentage of hæmoglobin is then read off on the tube.

CHEMISTRY OF HÆMOGLOBIN.

Hæmoglobin is a conjugated protein consisting of the protein, globin, in combination with the chromatogenic substance, hæmatin. Owing to the presence of this group it is very readily obtained in a crystalline state; the crystals vary in form according to the animal from whose blood they are prepared. Figs. 40-43 show the crystalline forms of hæmoglobin prepared from human, cat's, squirrel's, and guinea-pig's blood.

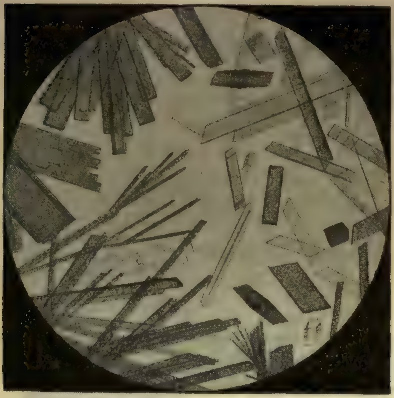


FIG. 40.—Human.

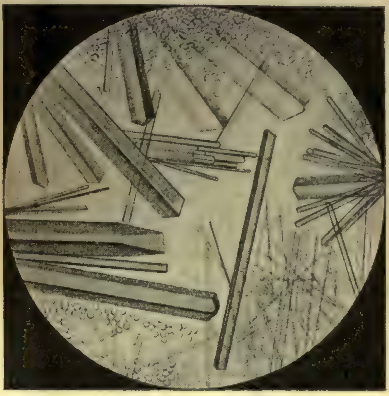


FIG. 41.—Cat.

(After Funke.)

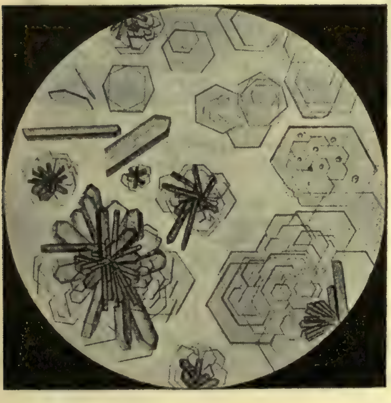


FIG. 42.—Squirrel.

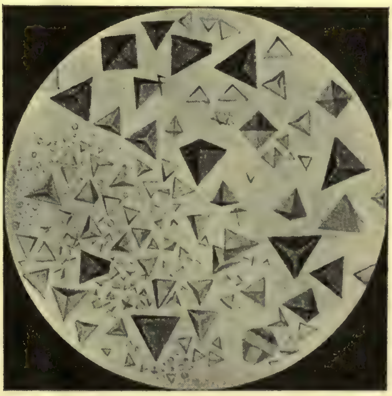


FIG. 43.—Guinea-pig.

(After Funke.)

The principal property of hæmoglobin is its power of combining with gases, especially oxygen and carbon monoxide. Whereas the oxygen compound, oxyhæmoglobin, readily dissociates into oxygen and hæmoglobin, the carbon monoxide compound, carboxyhæmoglobin, is very stable and can only be dissociated by prolonged treatment with oxygen. Cases of coal gas poisoning, due to the presence of carbon monoxide in the gas, are therefore generally fatal. Hæmoglobin also combines with nitric oxide, forming nitric oxide hæmoglobin, which is similar in properties to carboxyhæmoglobin.

Oxidizing agents convert hæmoglobin into methæmoglobin, which can be reconverted into oxyhæmoglobin.

The conjugated protein is very readily separated by the action of dilute acids or alkalies or by enzymes into its constituents, hæmatin and globin.

Hæmatin is soluble in acids and alkalies forming acid and alkali hæmatin. Reducing agents convert it into reduced hæmatin; reduced alkali hæmatin is known as hæmochromogen. Hæmatin is readily prepared by the action of glacial acetic acid on hæmoglobin. Its hydrochloride, hæmin, has a typical crystalline form (Fig. 44) and is often used as a test for blood stains.

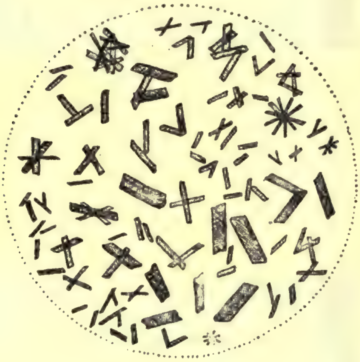


Fig. 44.—Hæmin crystals.

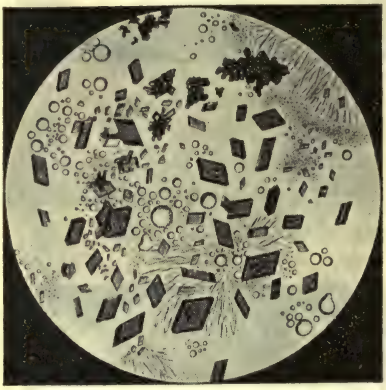


Fig. 45.—Hæmatoidin. (After Funke.)

Hæmatin contains iron in organic combination. When hæmatin is treated with strong acids—sulphuric acid, hydrobromic acid in glacial acetic acid—the iron is split off and hæmatoporphyrin (iron-free hæmatin) is obtained. The fact that hæmatin is only broken down by strong acids into iron and hæmatoporphyrin points to the combination of the iron with a carbon atom of hæmatoporphyrin. (Compare ferrocyanides.)

Another iron-free decomposition product of the colouring matter of the blood is hæmatoidin (Virchow), a substance which sometimes occurs in old blood-clots and which crystallizes in rhombic plates of an orange-red colour (Fig. 45).

Hæmatoidin is identical with bilirubin. The fact that it arises in old blood-clots and is identical with bilirubin proves that the pigments of the bile are derived from those of the blood.

1. Crystallization of Oxyhæmoglobin.

(*a*) From rat's blood. To a small quantity of rat's blood add an equal volume of water and 1 to 2 drops of ether, and shake thoroughly. Place a drop on a microscope slide, cover it, and examine under the microscope. Rhombic crystals gradually separate out.

(*b*) From sheep's, dog's, horse's, or guinea-pig's blood. To 20 c.c. of blood add 2 c.c. of water and 2 c.c. of ether; thoroughly mix until the blood is completely laked. This can be controlled by examining with the microscope. Then add an equal volume of saturated ammonium sulphate solution and thoroughly mix. Filter immediately from the precipitate and allow the filtrate to stand for about 24 hours in a corked flask. On examining the sediment with the microscope rhombic crystals will be seen, except in the case of guinea-pig's blood from which characteristic tetrahedral crystals are obtained.

Crystals of oxyhæmoglobin can be rapidly prepared from dog's blood as follows:—Add ether, drop by drop, to a little defibrinated dog's blood till the blood is completely laked. Now dissolve a pinch of finely powdered ammonium oxalate in it and allow to stand. Crystals of oxyhæmoglobin separate out, especially if the solution be cooled to 0° C. by immersion in ice. The crystals consist of thin rhombic prisms when examined under the microscope.

If the blood does not crystallize within an hour, spread out a drop on a microscope slide in a thin layer and leave it for five minutes. Cover with a slip and observe the crystals which form at the edges.

Methæmoglobin crystals can be obtained in the same way but the laked blood must first be treated with a few drops of potassium ferricyanide.

Crystals of hæmoglobin may be prepared in large quantities from horse's blood by Abderhalden's modification of Zinoffsky's method. The red blood corpuscles of horse's blood, after separating from serum and washing with 0·9 per cent. sodium chloride solution, are laked by mixing with twice their volume of water and warming to 35° C. Ammonia is added to dissolve the stromata of the corpuscles and it is then exactly neutralized with dilute hydrochloric acid. To this solution a quarter of its volume of alcohol is added and the mixture kept at 0° C. Crystals separate out on standing. These are filtered off, washed with alcohol and water in the above proportions and recrystallized by solution in two volumes of water, adding a quarter volume of alcohol and again cooling to 0° C.

2. Preparation of Hæmin Crystals.

(*a*) *Zeynek and Nencki's Method.* Precipitate the proteins from 10 c.c. of defibrinated blood by adding acetone. Filter off the precipitate and extract it with 10 c.c. of acetone acidified with 2 to 3 drops of hydrochloric acid. Place a drop of the dark-brown solution on a glass slide, cover and examine under the microscope. Minute crystals of hæmin separate out as the acetone evaporates. Larger crystals can be obtained by evaporating the acetone solution to half its volume, cooling and allowing to stand in a closed flask for 24 hours. Hæmin crystals will be found adhering to the bottom and sides of the flask. They have a bluish-black colour with metallic lustre and consist of long rhombic needles, arranged in rosettes and sheaves when examined under the microscope. If this sediment be filtered off and dissolved in caustic soda, the hæmin is decomposed and converted into hæmatin; if it be then reduced with ammonium sulphide or Stokes' reagent, hæmochromogen will be formed, which can be identified with the spectroscope. On acidifying with acetic acid, hæmatin is thrown out as a voluminous dark-brown precipitate.

(*b*) *Schalfejeff's Method.* Warm to 80° C. a little glacial acetic acid in a test tube. Then, stirring constantly, add not more than a quarter of its volume of defibrinated blood. The temperature falls to about 56° C. Warm again to 80° C. and allow it to stand for a short time. Examine a drop under the microscope for hæmin crystals. On allowing the remainder to stand for 24 hours, the hæmin crystals will settle out. Filter them off and wash them with water.

(*c*) *The formation of hæmin crystals is one of the best methods for testing for minute quantities of blood, blood-stains,* etc. Thus:—

Place a drop of blood on a glass slide and allow it to dry or heat it gently over a flame so as to drive off the water. Scrape the residue into a little heap and add a drop of glacial acetic acid from the end of a glass rod. Rub this up into a paste and put a little on a clean slide; add a drop of glacial acetic acid, cover with a cover-slip and heat till the solution begins to boil. Remove for a few seconds and heat again; repeat 2 or 3 times. Allow the slide to cool and examine under the microscope (high power). Observe the small black or brownish-black crystals of hæmin. (Teichmann's crystals.) Their appearance is shown in Fig. 44.

Dilute solutions of blood are acidified with acetic acid and precipitated with freshly prepared tannic acid. The dried precipitate is heated on a slide with a trace of sodium chloride and glacial acetic acid as described above. Blood-stains on cloth, linen, etc., are soaked in distilled water or weak alkali, examined with a micro-spectroscope, and the solution evaporated to dryness and treated as described. With old blood-stains it is necessary to add a crystal of sodium chloride; with fresh blood this is not essential.

3. Spectroscopic Examination of Hæmoglobin and its Derivatives.

When a beam of ordinary white light is passed through a prism it is resolved by the unequal refraction of its constituents at each bent surface into a band of light of several colours. This band of light is termed a spectrum, the colours being red, orange, yellow, green, blue, violet, indigo.

A spectrum obtained from a beam of sunlight shows a series of vertical dark lines—the Fraunhofer's lines. Thus in the red region three lines are seen; they are known as the A, B, C lines. In the yellow region one line, the D line, in the green three, the E, *b*, F lines; in the violet two, the G and H lines. These dark lines are due to the absorption of light in these regions of the spectrum by the passage of the light through certain volatile substances in the sun's atmosphere. The D line is due to sodium vapour as can be proved by passing ordinary light through sodium vapour when a line, or rather two lines very close together, are seen in the D position. The other lines are due to the vapours of other metals. The colour of light after passing through a coloured liquid is the colour, which is not absorbed by the liquid. Light when passed through a solution of hæmoglobin and then through a prism is absorbed in certain regions.

Hæmoglobin and its coloured decomposition products show typical absorption bands. The various compounds are therefore readily distinguished by means of a spectroscope.

see Haliburton

(*a*) **Oxyhæmoglobin.** Examine with a spectroscope a solution of 1 volume of defibrinated blood gradually diluted with 30 volumes of water. When concentrated, the solution appears dark red to the naked eye, but light will be found to pass through the red region of the spectrum between the Fraunhofer's lines C and D. On diluting further, light will pass through the green region, and between the red and green regions a dark absorption band will be seen. On diluting still further this band becomes resolved into two, the one lying nearer D being narrower than the other. It is immediately to the right of the sodium or D line, as can be seen by introducing a little sodium (e.g. common salt) into the flame when examined by gas-light. At the same time light will pass through the violet region.

Very dilute solutions of oxyhæmoglobin, yellowish-red to the naked eye, still show the two characteristic bands.

Note at what dilution the two bands are most clearly visible.

(*b*) **(Reduced) Hæmoglobin.** (1) Add a few drops of ammonium sulphide to a solution of oxyhæmoglobin, diluted so as to just show the two characteristic bands and warm to 50° C. after mixing. The colour becomes darker, and on examining with the spectroscope a single broad band will be seen between D and E.

(2) To a solution of oxyhæmoglobin as above, add 2 to 3 drops of Stokes' reagent.[1] This acts more rapidly than ammonium sulphide and need not be warmed. Observe the single absorption band of reduced hæmoglobin.

(*c*) **Carboxyhæmoglobin.** Pass a stream of coal gas (which contains CO) through a solution of oxyhæmoglobin. Note the peculiar cherry-red colour compared with the yellowish-red of oxyhæmoglobin. This is because less light in the blue and violet region of the spectrum is absorbed. Examine the solution with the spectroscope. Two bands like those of oxyhæmoglobin will be seen, but they are situated slightly nearer the violet end of the spectrum.

To a little of the solution add ammonium sulphide and warm, or Stokes' reagent. The two bands remain unaltered.

On adding half the volume of strong caustic soda, a light red precipitate is obtained. Normal blood, containing oxyhæmoglobin, when similarly treated gives a dark-brown coloration. The difference is less marked after standing.

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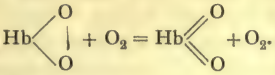
[1] Stokes' reagent consists of ferrous sulphate, tartaric acid and ammonia. It is prepared by dissolving 3 gm. ferrous sulphate in cold water, adding a cold solution of 2 gm. tartaric acid, and then making the mixture up to 100 c.c.; the ammonia is added when the reagent is required for use. Strong ammonia is then added till the precipitate first formed is redissolved. The solution rapidly absorbs oxygen and must therefore be freshly prepared. The solution of ferrous sulphate and tartaric acid can be kept for some time.

(*d*) **Methæmoglobin.** To some dilute blood add some concentrated potassium or sodium ferricyanide, and shake. The solution becomes reddish-brown in colour. Dilute a small portion and examine with the spectroscope. An absorption band to the red side of D will be seen, and the blue end of the spectrum is markedly absorbed. Dilute further; two faint bands appear in the green corresponding to those of oxyhæmoglobin.

Dilute another portion of the methæmoglobin solution and treat it with a few drops of ammonium sulphide. The colour becomes red and the single absorption band in the red disappears, giving place to the two bands of oxyhæmoglobin. In a short time these two bands will be replaced by the single band of reduced hæmoglobin.

When oxyhæmoglobin is converted into methæmoglobin a volume of oxygen equal to that originally combined with the oxyhæmoglobin is evolved. Methæmoglobin contains the same amount of oxygen as oxyhæmoglobin, but it is probably in a different state of combination, as it cannot be removed by submitting the methæmoglobin to a vacuum as in the case of oxyhæmoglobin. This change can be represented as follows:—



$$\mathrm{Hb}{<}\begin{matrix}\mathrm{O}\\ |\\ \mathrm{O}\end{matrix} + \mathrm{O_2} = \mathrm{Hb}{\ll}\begin{matrix}\mathrm{O}\\ \\ \mathrm{O}\end{matrix} + \mathrm{O_2}.$$

The evolution of oxygen in the conversion of oxyhæmoglobin into methæmoglobin can be readily observed. To a little defibrinated blood in a test tube add an equal volume of water and warm to 50° C. To this solution then add an equal volume of potassium ferricyanide solution and mix by shaking once. Incline the tube for a short time and observe the bubbles of oxygen which are evolved.

When treated with a reducing agent, the linkage of the oxygen atoms reverts to that of oxyhæmoglobin from which the oxygen can be removed by reducing agents.

Methæmoglobin is also obtained by the action of nitrites, potassium permanganate, potassium chlorate, hydrogen peroxide, e.g. add 2 drops of amyl nitrite to a solution of 1 in 5 of defibrinated blood, dilute and examine with the spectroscope.

In the body the formation of methæmoglobin may be the result of the action of certain aromatic substances, such as phenol.

If the solution of methæmoglobin be made faintly alkaline with a few drops of ammonia, alkaline methæmoglobin is formed. The solution is more red in colour and shows two bands between D and E, like those of oxyhæmoglobin, and a third more faint band to the red side of D.

(*e*) **Acid Hæmatin.** To a little diluted blood (1 : 5) add a quarter of its volume of 33 per cent. acetic acid. Mix thoroughly and warm in the water bath at 40 to 50° C. for 5 to 10 minutes. Dilute a small portion and examine with the spectroscope. An absorption band in the red between C and D will be seen. This is acid hæmatin. Make the solution faintly alkaline by gradually adding dilute caustic soda and filter. To the filtrate add a few drops of ammonium sulphide or Stokes' reagent. On examining with the spectroscope the two bands of hæmochromogen will be observed (*see below*).

Acid Hæmatin in Ethereal Solution. To a little defibrinated blood add about half its volume of glacial acetic acid and an equal volume of ether. Mix thoroughly. The ethereal solution of acid hæmatin rises to the surface; pour it off into a clean test tube and examine with the spectroscope. It shows a distinct band in the red region, and a broad band between D and F. Dilute with acid ether (1 part glacial acetic to 2 parts ether). The latter band is resolved into two, a narrow one in the light green to the red side of E, and a broader, darker one in the green. A fourth very faint band can be seen on the violet side of D.

Acid Hæmatin in Alcohol. Precipitate the proteins of some defibrinated blood by adding sufficient alcohol. Filter off the precipitate and extract it with alcohol containing 1 per cent. sulphuric acid. A dark-brown solution of hæmatin in acid alcohol is obtained.

(*f*) **Alkaline Hæmatin.** To a little diluted defibrinated blood (1 : 5) add half its volume of caustic soda, mix and heat gradually nearly to the boiling-point. The colour becomes brown. Cool and shake with air before examining with the spectroscope (reducing substances are formed by the action of the alkali and a portion of the alkaline hæmatin converted into hæmochromogen). A broad but very faint absorption band will be seen to the red side of D, extending a short distance towards the violet side. The blue end of the spectrum is considerably absorbed.

Alkaline Hæmatin in Alcohol. Mix a little defibrinated blood into a thin paste with solid potassium carbonate and evaporate it to complete dryness on the water bath. Powder the residue, boil it with alcohol in a flask on the water bath and filter. The solution contains alkaline hæmatin free from proteins and shows the absorption band of alkaline hæmatin more distinctly than the aqueous solution before.

(*g*) **Hæmochromogen.** To some of the aqueous solution of alkaline hæmatin add a few drops of Stokes' reagent or ammonium sulphide. The solution becomes red in colour and on spectroscopic examination shows two absorption bands in the green, the one nearer D being very prominent and sharply defined, the other being much fainter.

In very dilute solutions only the one band is seen but the absorption of light here is very great. A solution of oxyhæmoglobin, where the absorption bands can scarcely be seen, on conversion into hæmochromogen, will show this single band.

The conversion of hæmoglobin into hæmochromogen is a very delicate test for blood-stains; the stain is heated with 1 per cent. caustic soda, the solution is cooled and filtered, and then reduced with Stokes' reagent. Examination with the spectroscope shows one or both absorption bands.

(*h*) **Acid Hæmatoporphyrin.** To 5 c.c. of concentrated sulphuric acid in a test tube add 2 to 3 drops of defibrinated blood, and mix thoroughly after the addition of each drop. A purple solution will be obtained, which, when examined with the spectroscope, will show two well-marked absorption bands. The solution should be diluted with glacial acetic acid if necessary. One of the absorption bands lies in the orange between C and D, and the other, which is broader and darker, lies in the yellow-green between D and E.

(*i*) **Alkaline Hæmatoporphyrin.** Pour the greater part of the acid solution of hæmatoporphyrin into excess of distilled water. Cool the solution and nearly neutralize it with caustic soda. A pigmented precipitate separates out which contains most of the hæmatoporphyrin. Sodium acetate makes the precipitation more complete. Filter off the precipitate and dissolve it in dilute caustic soda. The solution of alkaline hæmatoporphyrin so obtained shows, when examined with the spectroscope, four absorption bands, a narrow one in the red, a broader and darker one in the green, a third in the green extending to the violet side of E, and a fourth at the junction of the blue and green. Hæmatoporphyrin when occurring in urine is always alkaline.

Laidlaw's Method for obtaining Hæmatoporphyrin.

Blood which has stood for 2 to 3 days in the cold, i.e. reduced by standing, is treated with one-third of its volume of concentrated hydrochloric acid and filtered. The mass of hæmatoporphyrin and precipitated proteins is extracted with alcohol; the alcoholic filtrate shows the acid spectrum of hæmatoporphyrin. On rendering just alkaline with ammonia the alkaline spectrum of hæmatoporphyrin may be observed. It is, however, best seen if the alcoholic solution be evaporated to dryness on the water bath and the pigment dissolved in dilute ammonia.

Hæmatoporphyrin is free from iron, whereas hæmatin still contains this element. The removal of the iron from hæmatin in the absence of reducing agents is accomplished only with difficulty, and is only effected by strong reagents such as concentrated sulphuric acid, glacial acetic acid saturated with hydrobromic acid or hydrochloric acid and heated to 130° C. in a sealed tube. The hæmatoporphyrin obtained by the sulphuric acid method is hæmatoporphyrin anhydride.

The iron is readily removed from hæmochromogen by dilute acids, and reducing agents of an acid nature easily set free hæmatoporphyrin from hæmatin. Hence the stability of iron in hæmatin is dependent on the presence of oxygen.

It is very probable that the cells of the body deal with the blood pigment in the reduced condition which, as seen above, is easily changed into hæmatoporphyrin. Its occurrence in the urine in cases of hæmatoporphyrinuria can be thus accounted for.

INTEGRATION OF THE CHEMICAL PROCESSES IN THE ANIMAL BODY.

METABOLISM.

Our knowledge of the complete course of the changes which the various chemical compounds undergo in their synthesis and degradation in both plants and animals is so insufficient that it is not possible to draw up a full and proper balance sheet.

If, however, the intake and output of the individual elements, carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur, etc., which are contained in the compounds, be ascertained, we can obtain some idea of the extent to which they are utilized. Thus, a known weight of carbohydrate or fat eaten by an animal is represented by the corresponding weight of carbon dioxide evolved by the lungs, unless some is retained in the body, in which case the body weight of the animal will increase. Similarly a known weight of protein or nitrogenous food will be represented by the corresponding weight of nitrogen in the urine. The actual amount of each element ingested by an animal is the amount consumed less the amount contained in the fæces. This consists of undigested food, i.e. food which has not entered into the circulation. When the intake and output of the elements is the same, then the animal is said to be in carbon, nitrogen, phosphorus, etc., equilibrium ; when less is put out than taken in then there is retention or assimilation and increase of body weight, when more, then there is loss of weight.

In addition to these factors, the energy factor can also be ascertained. Each foodstuff has a certain caloric value and can evolve on complete combustion a definite amount of heat. This serves to maintain the body temperature. The total heat evolved by the body corresponds to the heat value of the food.

The carbon-equilibrium is determined by the analysis of the inspired and expired air. The animal is placed in a suitable chamber, fed upon a given diet, the volume of gas which enters and which leaves is measured and samples of each are analysed. The carbon in the urine must also be estimated.

The energy equilibrium is determined in the same way except that the animal is placed in a special calorimeter through which air of a constant temperature can be passed, measured, and analysed.

The nitrogen, phosphorus, sulphur, etc., equilibrium is determined by the analysis of the urine. These analyses, in contradistinction to the above, are very easy to carry out and require no particular apparatus. A great deal of our present knowledge of dietetics is based upon such analyses, and valuable conclusions can be drawn from them.

The total amount of nitrogen in urine is the sum of the amounts of nitrogen contained in the various compounds, namely ammonia, urea, amino acids, uric acid, creatinine, indican, etc. The amount of nitrogen in the ammonia, urea, and amino acids corresponds to the amount of protein in the nitrogenous food. The uric acid nitrogen, according to the current beliefs, is derived partly from the purines in the food and partly from the purines in the organs of the body. It is very possible that some of the uric acid is derived by synthesis from ammonia or urea and a three-carbon atom complex as in birds. Its amount greatly increases under febrile conditions.

The creatinine nitrogen is doubtless derived from the creatine of muscle although the careful analyses of Folin, Cathcart, Hoogenhuyze and Verploegh fail to show the relationship between these bodies in metabolism ; whatever the diet, the daily creatinine output is always the same for any one individual. Excess of creatinine is excreted as creatinine, and excess of creatine is excreted as creatine, not as creatinine. The indican nitrogen is insignificant in amount in comparison with the others ; it shows the extent to which bacterial action takes place in the intestine.

There remains some 5 per cent. of nitrogen which cannot be accounted for and which is represented by pigments and unknown substances.

The chlorine corresponds exactly with the amount of salt eaten ; in certain diseases, e.g. pneumonia and cancer of the stomach, a retention of chlorides has been observed. The sulphur in urine is of three kinds : (1) inorganic sulphate, (2) ethereal sulphate, (3) neutral sulphur. The sulphates are derived mainly from the sulphur in the protein : the amount of sulphate in the food is very small. The ethereal sulphates increase in amount when putrefactive changes occur in the intestine or when poisonous substances, such as phenol, are taken. The neutral sulphur consists of cystine, thiocyanate, sulphides and other substances.

The phosphorus bears a direct relation to the amount of food ingested. Phosphorus compounds—phosphates, nucleic acid, lecithin, phosphoprotein—are present in all foods. The animal converts the organic compounds into inorganic phosphate. Only a small quantity of phosphorus is excreted in the urine in organic combination.

The following table gives the average daily output of the various constituents on a fixed carbohydrate and fat diet, but variable protein diet (egg-white, meat, etc.), the total quantity ingested corresponding to Voit's standard (see p. 170).

Diet.	Acidity $\frac{N}{10}$.	P_2O_5.	Ammonia.	Urea.	Creatinine.	Uric Acid.
	c.c.	gm.	gm.	gm.	gm.	gm.
Mixed	652	2·76	0·52	30·35	1·29	0·86
Meat	453	2·42	0·43	31·58	1·38	0·79
Egg-white	308	1·13	0·34	31·69	1·12	0·51
Egg-white + Na_2HPO_4	402	1·39	0·40	30·65	1·24	0·46
+ $CaCl_2$	331	0·73	0·65	28·83	0·97	0·50
+ lecithin	446	2·05	0·62	28·22	1·08	0·44
+ lemco	384	2·25	0·47	31·79	1·69	0·76
+ herring roe	778	4·44	0·86	29·19	1·27	1·16

CORRESPONDING NITROGEN VALUES OF THE ABOVE.

Total N.	Urea N.	Creatinine N.	Uric Acid.
gm.	gm.	gm.	gm.
16·01	13·91	0·52	0·29
17·09	15·01	0·50	0·26
16·23	14·75	0·42	0·17
15·91	14·30	0·46	0·16
15·10	13·46	0·37	0·17
14·80	13·15	0·41	0·15
16·56	14·50	0·63	0·26
16·53	13·62	0·48	0·41

AMOUNT OF NITROGEN IN PER CENT. OF THE TOTAL NITROGEN.

Ammonia.	Urea.	Creatinine.	Uric Acid.	Undetermined.
3·3	86·2	3·3	1·8	4·2
2·5	86·8	3·0	1·5	4·8
2·0	91·0	2·6	1·1	2·7
2·5	89·1	2·9	1·0	3·9
3·5	89·1	2·4	1·1	3·5
3·6	88·9	2·8	1·0	3·5
2·5	90·3	3·5	1·5	1·9
3·7	84·0	2·8	2·2	5·8

EXAMINATION AND ANALYSIS OF URINE.

1. **Colour.** The colour is of a transparent yellow, which varies from light yellow to orange or red-brown; the froth which forms on shaking soon disappears. It contains no sediment.

2. **Reaction.** The reaction is acid to litmus paper. This acidity is due to acid phosphates and not to free acid. At the full height of digestion it may be slightly alkaline and may show a cloudiness due to the presence of phosphates. It becomes alkaline on standing owing to the setting in of "ammoniacal fermentation" by the *micrococcus ureae*, which converts urea into ammonium carbonate. This change is in reality due to the action of the enzyme—urease—liberated from the micro-organism.

3. **Specific Gravity.** The specific gravity varies from 1010-1025. The average is 1017-1020. Take the specific gravity with a hydrometer (urinometer).

4. **Volume.** Its volume varies. The average volume is 1500 c.c. per day.

It is very advantageous to make the volume up to 2000 c.c. after taking the specific gravity before carrying out the analyses.

5. Estimation of the Acidity and "Ammonia".

In order to estimate the acidity a sufficient amount of alkali of known strength is added from a burette until neutralization is produced, as indicated by the colour reaction of phenolphthalein, which has been previously added.

It must be remembered that different indicators give different results. Congo red and methyl orange are only affected by strong acids, whereas litmus and phenolphthalein are affected by weak acids, such as carbonic acid.

The estimation of ammonia in urine until recent years has been a matter of considerable trouble on account of the length of time required to perform an analysis. Folin in 1902 employed a strong current of air to drive off the ammonia from urine made alkaline with sodium carbonate, a process which required at least 1½ hours, and Malfatti in 1908 brought forward the following method which can be carried out very rapidly and is very accurate in its results. It depends upon the fact that when a neutral solution of an ammonium salt is treated with formaldehyde, combination occurs with the formation of hexamethylene tetramine (urotropin) and the liberation of a corresponding amount of acid which ~~can be titrated~~ with $\frac{N}{10}$NaOH. The reaction is:—

$$4NH_4Cl + 6CH_2O + 4NaOH = N_4(CH_2)_6 + 10H_2O + 4NaCl.$$

The value so obtained is higher than the value obtained by the Folin method, and is due to the presence of amino acids in the urine which react with formaldehyde in the same way as ammonia (see p. 107). The result is therefore the amount of ammonia and amino acids.

The standard solutions employed are normal solutions of sulphuric acid and caustic soda diluted to $\frac{1}{10}$ strength and called $\frac{N}{10}H_2SO_4$ and $\frac{N}{10}NaOH$ respectively. Normal solutions contain the molecular weight or gramme equivalent of the substance in a litre of the solvent, e.g. 36·5 gm. HCl, 40 gm. NaOH. As a molecule of H_2SO_4 can neutralize two molecules of NaOH its normal solution contains $\frac{98}{2}$ or 49 gm. in a litre.

The starting-point for making a standard solution is oxalic acid which can be obtained pure and dry. 6·3 gm. of pure crystalline oxalic acid, $(COOH)_2 . 2H_2O$ are dissolved in water and the solution made up to a litre.

Standard $\frac{N}{10}NaOH$ is prepared by dissolving about 4 gm. of pure caustic soda in water and making up to a litre. This is then standardized by titrating against the standard oxalic acid solution. 20 c.c. of the $\frac{N}{10}$ oxalic acid solution are placed in a flask, a few drops of methyl orange are added and the NaOH solution is slowly run in from a burette till the solution just becomes yellow, the mixture during this time being well shaken. The exact strength of the NaOH solution is thus ascertained. It must then be diluted so that 20 c.c. $\equiv$ 20 c.c. $\frac{N}{10}$ oxalic acid.

Standard $\frac{N}{10}H_2SO_4$ is prepared by titrating 5 or 10 c.c. of dilute H_2SO_4 with the standard $\frac{N}{10}NaOH$ in the same way and then diluting so that 20 c.c. $\equiv$ 20 c.c. $\frac{N}{10}NaOH$.

Method.

Standardize the approximately $\frac{N}{10}$NaOH by titrating it with the given standard $\frac{N}{10}$ acid, and then use this solution to estimate the acidity of the given urine as follows:—

Measure out 25 c.c. of urine with a pipette into a flask or beaker and add 50 to 100 c.c. of water. Add 6 drops of phenolphthalein and run in the standard $\frac{N}{10}$NaOH till the solution has a permanent and distinct pink colour.[1]

acid

The indistinctness of the end point is due to the presence of calcium salts and ammonium salts. Folin therefore recommends that the titration be carried out in the presence of potassium oxalate, which diminishes the error. Thus:—

Dilute 25 c.c. of urine with an equal volume of water, add 15 gm. of finely-powdered potassium oxalate and 4 or 5 drops of phenolphthalein. Shake thoroughly for 1 to 2 minutes and, whilst the solution is still cold from the effect of the oxalate, titrate with $\frac{N}{10}$NaOH until a permanent pink tint remains. Note the number of c.c. added.

Now add 10 c.c. of formalin diluted with two volumes of water and neutralized to phenolphthalein with $\frac{N}{10}$NaOH. The colour disappears. Run the $\frac{N}{10}$NaOH into the mixture until a permanent pink colour is again obtained. Carefully note the number of c.c. added.

am

Calculation of Results.

The acidity result is expressed in terms of $\frac{N}{10}$NaOH either as percentage or per 24 hours' quantity. Suppose 2·5 c.c. of $\frac{N}{10}$NaOH are required for 10 c.c. urine, the acidity of the urine (100 c.c.) is equivalent to 25 c.c. of $\frac{N}{10}$NaOH; or, calculated for the 24 hours' quantity of urine, it is $2·5 \times \frac{2000}{10}$ if the urine has been diluted to 2000 c.c.

The "ammonia" result is expressed in gm. per 24 hours. Thus, if 6 c.c. $\frac{N}{10}$NaOH were used with 25 c.c. urine, then

$$\text{As } 6 \text{ c.c. } \frac{N}{10}\text{NaOH} = 6 \text{ c.c. } \frac{N}{10}\text{NH}_3 = 6 \times 0·0017 \text{ gm. NH}_3,$$

$$\frac{2000}{25} \times 6 \times 0·0017 \text{ gm. NH}_3 = \text{amount of "ammonia" in 24 hours.}$$

N.B

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[1] Only approximate results are given by this method, owing to the difficulty of determining the end point (compare with pure aqueous solutions as used in the standardization). The end point varies with the amount of indicator used, so that this must be kept constant. Each worker should choose his own end point and always proceed to this point, and then reliable information can be obtained concerning the relative acidity of several urines.

Na Cl. Sol

6. Estimation of Chlorides by Volhard's Method.

The principle of this method consists in precipitating the chlorides by excess of a standard solution of silver nitrate in the presence of nitric acid. The excess of silver is then estimated in an aliquot part of the filtrate with a solution of potassium or ammonium thiocyanate, which has been previously standardized against the silver solution, a ferric salt being used as indicator.

The following solutions are required :—

1. Standard silver nitrate solution either $\frac{N}{10}$, or of such a strength that 1 c.c. corresponds to 0·01 gm. NaCl.
2. Potassium thiocyanate solution (8 gm. per litre).
3. Pure nitric acid free from chlorides.
4. A saturated solution of iron alum.

The potassium thiocyanate solution must be standardized against the silver nitrate solution.

Method.

1. 1. **Standardization of the Thiocyanate Solution.** Place 10 c.c. of the silver nitrate solution with a pipette in a beaker, add 5 c.c. of pure nitric acid, 5 c.c. of iron alum solution and 80 c.c. of water. Now run in the thiocyanate solution from a burette until a permanent red tinge is obtained. Note the amount required for the 10 c.c. silver nitrate solution.

2 ple of chlorides

2. **Analysis.** Place 10 c.c. of urine in a 100 c.c. measuring flask with a pipette. Then add about 4 c.c. of pure nitric acid and 20 c.c. with a pipette of the standard silver nitrate solution. Now fill up to the mark with distilled water, mix thoroughly and filter into a dry vessel through a dry paper.

) titrate back excess of $AgNO_3$.

Take exactly 50 c.c. of the filtrate with a pipette and titrate it with the thiocyanate solution until a permanent red colour is obtained, iron alum having been added before the titration is commenced.

Calculation of the result :—

50 c.c. filtrate = S c.c. KCNS

$AgNO_3$ cc excess ∴ 100 c.c. „ = 2S c.c. KCNS.

Now x c.c. KCNS = 10 c.c. $AgNO_3$

$$\therefore \text{ 2S c.c. ,, } = \frac{10 \times 2S}{x}.$$

This is the excess not utilized to precipitate the chlorides

$$\therefore \left(20 - \frac{10 \times 2S}{x}\right) = \text{amount of } AgNO_3 \text{ solution used.}$$

As $\frac{N}{10}$ $AgNO_3$ = $\frac{N}{10}$ NaCl, 1 c.c. solution = 0·0585 gm. NaCl.

Hence NaCl in gm. in the volume passed in 24 hours.

Query. Why cannot chlorides be estimated directly in urine?

7. Estimation of Phosphates.

Sol
phosp

The method depends upon the precipitation of all the phosphates by a standard solution of uranium acetate or uranium nitrate (in the presence of sodium acetate and acetic acid) as uranium phosphate, $(UrO_2)HPO_4$:—

$$UrO_2(C_2H_3O_2)_2 + KH_2PO_4 = UrO_2HPO_4 + KC_2H_3O_2 + C_2H_4O_2.$$

The determination of the point, when excess of soluble uranium salt is in solution, is shown by means of potassium ferrocyanide, or by cochineal tincture, which becomes green at this point.

The following reagents are required:—

1. Acid sodium acetate solution.
2. Cochineal tincture.
3. Standard uranium solution $\frac{N}{10}$, or so that 1 c.c. = 0·005 gm. P_2O_5 or 5 mgm.

This must be prepared by standardizing against a standard phosphate solution. Generally sodium phosphate is employed; 12 gm. are weighed out and dissolved in 1000 c.c. of water; 50 c.c. of this solution are evaporated to dryness, incinerated and weighed as pyrophosphate. From the weight of this the amount of P_2O_5 in 50 c.c. can be calculated and the remainder of the solution can be diluted, so that 50 c.c. contain 0·1 gm. P_2O_5. It is simpler to use acid potassium phosphate, KH_2PO_4, which can be weighed out directly and dissolved in water, so that 50 c.c. contain 0·1 gm. P_2O_5. 50 c.c. of this solution are titrated with the uranium solution (36 gm. in 1 litre) in the manner described below, and the uranium solution is then diluted so that 1 c.c. = 5 mgm. P_2O_5.

Method of Analysis. Place 50 c.c. of urine with a pipette in a 100 c.c. beaker, add 5 c.c. of acid sodium acetate solution and a few drops of cochineal tincture. Heat the urine to boiling and run in slowly the standard uranium acetate solution from a burette as long as a precipitate is formed. Keep the solution boiling and add the uranium solution, drop by drop, until the red colour is changed to green. This end point is best tested by taking out a drop of the solution and placing it in contact with a drop of potassium ferrocyanide solution, or a little heap of finely powdered ferrocyanide, on a white piece of porcelain. A brown colour or precipitate is formed when excess of soluble uranium salt is present in the solution. (A few more drops are generally required to reach this point than to turn the cochineal green.)

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Calculation of the Result:—

50 c.c. of urine = n c.c. of uranium solution = $n \times 0{\cdot}005$ gm. P_2O_5. Hence the quantity of P_2O_5 in the 24 hours' quantity of urine.

The average daily excretion of P_2O_5 is 2·5 gm.

8. Estimation of Sulphates.

These are of two kinds, inorganic and ethereal or conjugated. It is often of great importance to know their relative amounts. This amount is generally determined by difference either between the total amount and the amount of inorganic sulphate which is thrown down in acetic acid solution, by which the conjugated sulphate is not hydrolyzed, or between the total amount and the amount of the ethereal sulphate. The inorganic sulphate is in this case precipitated by a mixture of barium chloride and hydrate, filtered off, and the ethereal sulphate hydrolyzed by hydrochloric acid and estimated. The most accurate method is gravimetric, but Neubauer has suggested the following volumetric method.

Volumetric Estimation of Total Sulphates.

This method consists in adding standard barium chloride solution to a known volume of urine so long as barium sulphate is precipitated.

The standard barium chloride solution is $\frac{N}{10}$, or it contains 30·5 gm. $BaCl_2 . 2H_2O$ in a litre of water, so that 1 c.c. = 0·01 gm. SO_3.

.005 H_2SO_4 =
.00

10 per cent. potassium sulphate solution and pure hydrochloric acid are also necessary.

Method. Measure out 100 c.c. of urine with a pipette into a flask. Acidify it with 5 c.c. of pure hydrochloric acid and boil gently for 15 minutes so as to convert the ethereal sulphates into inorganic sulphates. Then from a burette allow the barium chloride solution to run in as long as a precipitate forms, the mixture being heated before every addition of barium chloride. Allow the precipitate to settle when 5 to 8 c.c. of barium chloride solution have been run in. Pipette off a few drops of the supernatant liquid into a watch glass on a black surface and add a few drops of the barium chloride solution. If there is a precipitate, return the whole to the flask washing in the last traces with water and add more barium chloride. Again allow to settle and test as before. Proceed until no more barium sulphate is precipitated.

Excess of barium chloride must be avoided. When there is only a trace a drop of the clear fluid removed from the flask will only give a cloudiness with a drop of potassium sulphate solution placed in a watch glass on a dark ground. If more than a cloudiness is produced, excess has been added and the whole operation must be repeated.

Calculate the percentage of the SO_3 in the urine, or in the 24 hours' quantity.

The average amount is 2·5 gm. per day. Its amount varies directly with that of the nitrogen. The value $\frac{N}{H_2SO_4}$ is 5.

9. Estimation of the Total Nitrogen.

This estimation is carried out by Kjeldahl's method as described on p. 20. 5 c.c. of urine are heated with 10 c.c. of pure sulphuric acid and a tiny crystal of copper sulphate till oxidation is complete.

The ammonia formed is distilled off and collected in 50 c.c. $\frac{N}{10}$ H_2SO_4.

Titration with $\frac{N}{10}$ NaOH gives the amount of $\frac{N}{10}$ NH_3 in 5 c.c of urine.

Hence the amount of nitrogen in the 24 hours' quantity, e.g.

Suppose 100 c.c. $\frac{N}{10}$ H_2SO_4 were taken and 66 c.c. $\frac{N}{10}$ NaOH used.

Difference $= 34$ c.c. $\frac{N}{10}$ $H_2SO_4 = 34$ c.c. $\frac{N}{10}$ $NH_3 = 34$ c.c. $\frac{N}{10}$ N.

$= 34 \times 0{\cdot}0014$ gm. N in 5 c.c. urine.

Hence the amount of N in the 24 hours' quantity.

10. Estimation of Urea.

The most accurate method of estimating urea is that of Folin by the hydrolysis of the urea into ammonia by hydrochloric acid.

The most rapid method is the hypobromite method as described on p. 112. 5 c.c. of urine are treated with 25 c.c. of hypobromite solution and the volume of nitrogen evolved is measured.

Other substances such as ammonia, creatinine, uric acid, evolve nitrogen under these conditions but it is very small in comparison. The method gives good comparative values.

As 354 c.c. of nitrogen at 0° C. and 760 mm. pressure are evolved by 1 gm. of urea, the amount of urea in 5 c.c. of urine can be calculated. Hence the amount in the 24 hours' volume.

11. Estimation of Uric Acid.

In 1892 it was shown by Hopkins that uric acid could be completely precipitated from urine as ammonium urate by saturating the urine with ammonium chloride. Its amount was estimated by converting this into uric acid by the action of hydrochloric acid, dissolving the latter in sodium carbonate and titrating with standard permanganate solution.

Wörner in 1900 shortened the process by heating the precipitate of ammonium urate, washed free from chlorides with ammonium sulphate, with caustic soda until all the ammonia was evolved, and then estimating the nitrogen by Kjeldahl's method. The results so obtained have been found to be not so accurate as those obtained by Hopkins' method.

Very accurate results are obtained by Folin and Schaffer's method, which is in reality a shortened Hopkins' method, and is the one most commonly employed.

For this method the following reagents are required:—

1. A solution of ammonium sulphate, uranium acetate, and acetic acid made up as follows: 500 gm. ammonium sulphate, 5 gm. uranium acetate, and 60 c.c. 10 per cent. acetic acid are dissolved in 650 c.c. water. The volume of this solution is almost exactly 1000 c.c.

Folin-
chaffer's
eagent

2. 10 per cent. ammonium sulphate solution.

3. $\frac{N}{20}$ potassium permanganate solution made by dissolving 1·581 gm. pure potassium permanganate in 1 litre of water. 1 c.c. = 0·00375 gm. uric acid.

Method. Measure out 200 c.c. of urine with a pipette into a 500 c.c. flask and add 50 c.c. of the ammonium sulphate and uranium acetate reagent. Mix the solutions and allow to stand for about half an hour so as to allow the precipitate to settle. This precipitate contains a mucoid substance which, if not thus removed, renders the subsequent filtration and washing of the ammonium urate precipitate very slow. Filter off the supernatant liquid through a dry filter into a dry vessel, and measure out 125 c.c. (= 100 c.c. urine) of this with pipettes into a beaker. Add 5 c.c. of concentrated ammonia, mix well and allow it to stand covered with paper for 12 to 24 hours.

Folin
Shaffer

Carefully decant the supernatant liquid upon a filter, wash the precipitate of ammonium urate on to the filter with 10 per cent. ammonium sulphate solution and then wash once or twice with the same reagent to remove the chlorides as completely as possible.

Remove the filter from the funnel, open it and with a fine stream of water wash the ammonium urate precipitate into a beaker. To the ammonium urate precipitate suspended in about 100 c.c. of water add 15 c.c. of concentrated sulphuric acid and titrate at once without cooling with the potassium permanganate solution.[1] At first every small addition of the permanganate is decolorized before it diffuses through the liquid, but towards the end the decolorization is slower and the permanganate should be added two drops at a time until a faint pink colour is seen throughout the whole solution (cf. p. 118).

N. p
v. u

Calculation of the result :—

$$1 \text{ c.c. } KMnO_4 = 0{\cdot}00375 \text{ gm. uric acid,}$$
$$\therefore x \text{ c.c. } ,, = x \times 0{\cdot}00375 \text{ gm. uric acid.}$$

A correction of 0·003 gm. or 3 mgm. for every 100 c.c. urine used must be added on account of the solubility of ammonium urate :—

$$\therefore 100 \text{ c.c. urine contain } x \times 0{\cdot}00375 + 0{\cdot}003 \text{ gm. uric acid.}$$

Hence the amount in the 24 hours' quantity.

12. Estimation of Creatinine.

This is carried out by Folin's adaptation of the Jaffé colour reaction of creatinine with picric acid and caustic soda. Folin found that a layer 8 mm. deep of $\frac{N}{2}$ potassium bichromate had the same colour as a layer 8·1 mm. deep of a solution prepared from 10 mgm. of pure creatinine, picric acid and caustic soda.

Method. 10 c.c. of urine are measured out with a pipette into a 500 c.c. measuring flask, 15 c.c. of saturated picric acid solution (12 gm. per litre) and 5 c.c. of 10 per cent. caustic soda are added and the mixture is allowed to stand for five to six minutes. The volume is then made up to 500 c.c. and the colour of this solution is at once compared with that of the standard bichromate solution. This solution has meanwhile been placed in one of the divisions of the colorimeter and the scale on this side of the instrument adjusted so that a layer 8 mm. deep is being looked through. The comparison is effected by altering the depth of the unknown solution by means of the screw on this side of the colorimeter until a position is found where the colours of the two solutions are properly matched.

Calculation of the result.

Suppose 9·5 mm. of the unknown solution match the 8 mm. of the bichromate solution, then

$$10 \text{ c.c. urine contain } 10 \times \frac{8{\cdot}1}{9{\cdot}5} = 8{\cdot}4 \text{ mgm. creatinine.}$$

Hence the amount in the 24 hours' quantity of urine.

13. Estimation of Indican.

The indican is converted into indigo-blue by Obermayer's reagent (see p. 143) and extracted with chloroform. The colour of this solution is compared with that of a standard indigo-blue solution. Folin has proposed that the comparison be made with Fehling's solution.

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[1] Rubber is attacked by potassium permanganate and it must therefore be run in from a burette with a glass tap.

APPENDIX.

I. THE PIGMENTS OF THE URINE.

(1) The chief pigment of the urine is urochrome, which gives urine its yellow colour.

(2) Urobilin is present only in small quantities, generally in the form of its chromogen, urobilinogen. Under certain pathological conditions its amount may be greatly increased. Urobilin is derived from haematin and is very like hydrobilirubin, which is obtained by reduction of bilirubin. It is identical with stercobilin, the yellow pigment of the fæces.

(3) Uroerythrin gives the pink colour to urate sediments. Normally it is present only in small amounts, but is greatly increased in certain diseases.

(4) Haematoporphyrin is also only present in small amounts.

I. *Urochrome and Urobilin in Normal Urine.*

Observe that normal urine shows no absorption bands with the spectroscope but only a general absorption of the violet. Neither urochrome nor urobilinogen shows absorption bands.

Saturate some urine with ammonium sulphate crystals[1] and allow it to stand a short time. Filter off the precipitate, which consists of ammonium urate associated with the chromogen of urobilin. Extract it with hot alcohol and note that the alcohol shows no absorption band or only a very faint one. Acidify the extract; the chromogen is broken up and the absorption band of urobilin can be seen at the junction of the green and blue.

To the filtrate containing the ammonium sulphate add 2 to 3 times its volume of alcohol. The ammonium sulphate is precipitated and a clear yellow layer of alcohol containing the urochrome forms above the salt solution. Examine with the spectroscope and note that there are no absorption bands.

---

[1] 80 gm. per 100 c.c.

II. *Pathological urine containing excess of urobilin* (*or normal urine*).

Precipitate the urates by saturating the urine with ammonium chloride (27 gm. per 100 c.c.) and filter. Saturate the filtrate with ammonium sulphate and add a drop of sulphuric acid. Shake it up with a mixture of two parts ether and one part chloroform. Note that the urobilin is taken up by this solvent and shows the absorption band well.

Pipette off the ether-chloroform layer into another test tube and shake well with water made slightly alkaline with caustic soda. The pigment passes entirely into the alkaline solution.

A solution of urobilin in alcohol shows a green fluorescence and an absorption band in the green between b and F.

III. *Pathological urine containing a pink urate sediment.*

Filter off the urate sediment and note its colour which is due to uroerythrin.

To a portion of this precipitate add caustic soda; the pink colour becomes green.

Dissolve the remainder of the precipitate in hot water, extract it with acetic ester or amyl alcohol. A pink solution is obtained which shows two absorption bands in the green; if weak, only one.

IV. *Hæmatoporphyrin in normal urine.*

For the detection of hæmatoporphyrin in normal urine, where it is present as alkaline hæmatoporphyrin, at least 200 to 400 c.c. are necessary, but in pathological urine, where its quantity is increased, a smaller quantity can be used. The best method is that of Garrod. To every 100 c.c. of urine add 20 c.c. of dilute caustic soda. This precipitates the earthy phosphates which carry down the pigment. The urine must give a precipitate like that of normal urine, otherwise calcium phosphate in acetic acid solution must be added before precipitating. This is obtained by precipitating a little calcium chloride with sodium phosphate in a test tube. Avoid too large a precipitate. Allow the precipitate of earthy phosphates to settle, pour off the supernatant liquid, transfer the precipitate on to a filter paper and wash it with water (till the washings are colourless if hæmatoporphyrin free from other pigments be required; avoid too much washing if only a little hæmatoporphyrin be present). Extract the precipitate with alcohol acidified with hydrochloric acid and examine for the spectrum of acid hæmatoporphyrin.

2. URINARY SEDIMENTS.

Separate the sediment thoroughly by means of the centrifuge, and examine it under the microscope.

A. IN ACID URINE.

Amorphous Deposits.

1. Urates.

This deposit is known as brick dust deposit from its pink colour (uroerythin). It may contain crystalline forms, and may dissolve completely on warming the urine.

Boil a little of the sediment with water. It is soluble ; whilst hot, acidify with hydrochloric acid. Cool. Examine the sediment of uric acid which forms with the microscope and test it with the murexide reaction. Urates may deposit from concentrated urine on cooling, as is commonly found in fevers.

The chief constituent is acid sodium urate.

2. Calcium Oxalate.

Calcium oxalate has the appearance of dumb-bell or spheroidal bodies (envelope crystals) under the microscope. It is insoluble in strong acetic acid and ammonia, but soluble in hydrochloric acid.

3. Bilirubin or Hæmatoidin.

Yellow granular amorphous masses which give Gmelin's reaction.

Crystalline Deposits.

4. Uric Acid.

This is known as cayenne pepper deposit as it is of sandy red colour. It has a distinctive crystalline form when examined with a microscope and gives the murexide reaction.

5. Calcium Oxalate.

This sediment consists of colourless, transparent, highly refractive octahedral crystals (envelope shaped). It is insoluble in acetic acid, but soluble in hydrochloric acid.

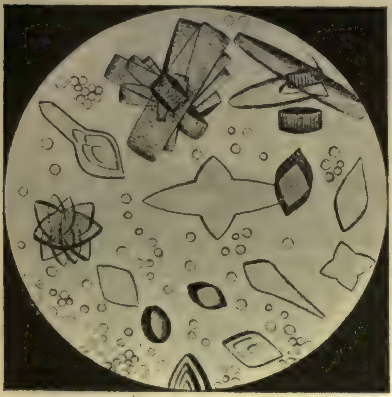


FIG. 46.—Deposit of uric acid.

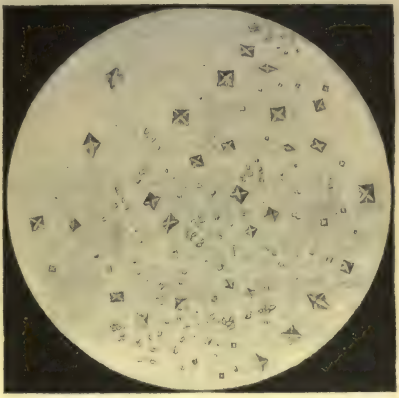


FIG. 47.—Deposit of calcium oxalate.

(After Funke.)

6. Ammonium Magnesium Phosphate.

Ammonium magnesium phosphate crystals separate in the shape of knife-rests from faintly acid urine. They are soluble in acetic acid.

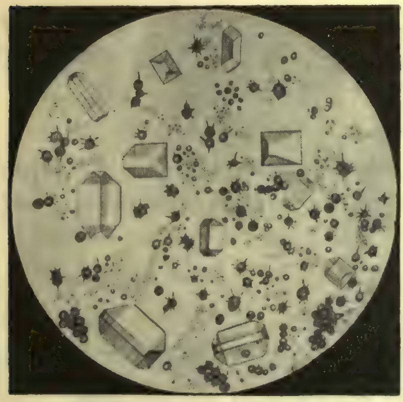


FIG. 48.—Deposit of triple phosphate (am. mag. phosphate) and ammonium urate.

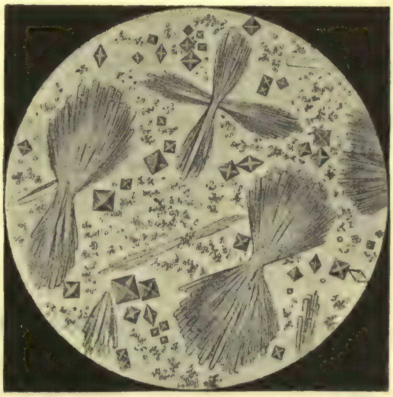


FIG. 49.—Deposit of uric acid, sodium urate and calcium oxalate.

(After Funke.)

The smaller crystals may resemble calcium oxalate in appearance, but are distinguished by their solubility in acetic acid.

7. **Calcium Hydrogen Phosphate.** $CaHPO_4 . 2H_2O$.

Large prismatic crystals, often arranged in rosettes. On adding a solution of ammonium carbonate, the crystals are eaten into and break down into an amorphous deposit. They are easily soluble in dilute acetic acid.

8. **Bilirubin or Hæmatoidin.**

Small yellow rhombic plates which give Gmelin's reaction.

9. **Tyrosine.**

Fine needles in star-shaped bundles (p. 134). They are insoluble in acetic acid, but soluble in ammonia and hydrochloric acid and give Millon's reaction.

10. **Cystine.**

Cystine deposits are very rare and consist of colourless regular hexagonal plates (p. 105), which are soluble in ammonia, but insoluble in acetic acid.

B. IN ALKALINE URINE.

Amorphous.

1. **Earthy Phosphates.**

Fine granules easily soluble in dilute acetic acid.

2. **Calcium Carbonate.**

Fine granules or dumb-bell shaped spheroidal masses sometimes having a concentric striation. They are easily soluble in acetic acid with evolution of carbon dioxide and effervescence.

3. **Acid Ammonium Urate.**

Spherules with small crystals adhering = hedgehog spines. They are generally pigmented, and dissolve in hydrochloric acid from which uric acid separates.

Crystalline.

4. **Ammonium Magnesium Phosphate.**

Large colourless prisms of knife-rest or coffin-lid shape. They are easily obtained from urine undergoing ammoniacal fermentation.

5. **Crystalline Calcium Phosphate.** $CaHPO_4$.

Occurs rarely, and appears as rosettes of prisms or spherules.

3. URINARY CALCULI.

The various constituents of urine which form sediments may take part in the formation of urinary calculi, which are of frequent occurrence and vary in size from that of a pea to that of a goose egg. They are generally coloured, commonly yellow-grey, yellow-brown or reddish-brown. Their surface may be smooth and polished, or rough and uneven. When fractured, they appear to consist of regular concentric layers which can often be scaled off.

1. Urate Stones.

They consist generally of ammonium urate and are of a brown colour and very hard; when powdered they form a pale yellow powder. They give the murexide reaction; ammonia is evolved on boiling them with caustic soda.

2. Calcium Oxalate Stones.

The most frequent in occurrence after the urate stones. They are either smooth and small (hempseed stones) or as large as a hen's egg with a rough and uneven surface (mulberry stones). They cause hæmorrhage and have therefore a superficial dark brown colour. They are soluble in hydrochloric acid without effervescence, but not in acetic acid. On heating they are converted into calcium carbonate, which dissolves in acetic acid with effervescence.

3. Phosphate Stones.

These stones can reach a large size. They consist of calcium phosphate and triple phosphate and generally contain ammonium urate and calcium oxalate. Their colour varies: it may be white, grey, pale yellow or even lilac (from indigo-red). Their surface is uneven. They do not burn when heated; when crushed the powder is soluble in acids without effervescence and the solution gives the reactions for phosphoric acid and calcium. Ammonia is evolved when stones containing triple phosphate are heated with caustic soda.

4. Calcium Carbonate Stones.

These stones occur chiefly in herbivora and very rarely in man. They have a chalky appearance, are white in colour, and dissolve in acids with evolution of carbon dioxide.

5. Cystine Stones.

These stones are rare and of various sizes. They may be as large as a hen's egg. Their surface is smooth or uneven, white or dull yellow and they show a crystalline fracture; they are not very hard. When heated they burn away almost entirely with a blue flame. They give the reaction for cystine (p. 107).

6. Xanthine Stones.

These stones are still rarer and vary in size from a pea to a hen's egg. In colour they are dull white, yellow-brown, or red-brown. They are not very hard, and they show an amorphous fracture; they become waxy on rubbing. They are completely combustible and give a yellow murexide reaction.

7. Urostealiths.

These stones have only been observed a few times. They are soft and elastic when wet, brittle, amorphous and waxy when dry. They burn with a luminous flame when heated. They consist of fat and cholesterol.

4. GALL STONES.

Calculi of various sizes and shapes and of variable number occur in the gall bladder. Three kinds have been distinguished:—

1. Pigmented Chalk Stones.

In man these stones are small; in the ox and pig stones as large as a walnut have been found. They consist almost entirely of the calcium salt of bilirubin and contain no, or very little, biliverdin. Sometimes black or greenish-black metallic-looking stones, which consist of bilifuscin and biliverdin, occur. Iron and copper are generally present. They are heavier than water.

2. Cholesterol Stones.

The shape and size of the cholesterol stones are very variable and they are generally lighter than water. Their fractured surface appears crystalline and they are composed of concentric layers. Their cut surface is waxy and their fractured surface when rubbed with the nail has a waxy appearance. By rubbing against one another in the gall bladder their appearance is generally faceted. They are almost white and usually show pigmented edges (pigmented chalk).

3. Calcium Carbonate and Phosphate.

These are very rare in man.

5. INBORN ERRORS OF METABOLISM.

Under this title A. E. Garrod has grouped together four anomalies in metabolism, namely albinism, alkaptonuria, cystinuria, pentosuria. These anomalies are of life-long persistence and in most cases are inborn. They may occur as temporary phenomena in disease.

1. Albinism.

The essential phenomenon of albinism is the absence of pigments of the melanin group which play the chief part in the coloration of man and the lower animals. All varieties of melanins are absent in albinos—white hair, pink eyes, and unpigmented skin.

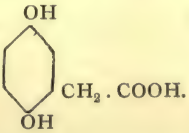
The lipochrome pigments which colour fats, serum, etc., yellow are not absent.

2. Alkaptonuria.

This is the best-known example of an inborn error. It is rendered evident by the freshly passed urine of an alkaptonuric beginning to darken on exposure to the air due to absorption of oxygen. The darkening commences at the surface and gradually spreads through the liquid until it assumes a black colour. Alkali greatly hastens the darkening.

The urine, when treated with Fehling's solution, gives a deep brown colour and there is a copious reduction. The colour of the liquid differentiates it from the reduction produced by other substances. Ammoniacal silver nitrate solution is rapidly reduced in the cold. Nylander's reagent is darkened, but there is no reduction to bismuth. A yellow precipitate is formed when such urine is treated with Millon's reagent. The most striking reaction is given with ferric chloride. If the reagent be allowed to fall, drop by drop, into the urine, a momentary deep blue colour appears; this can be repeated until oxidation is complete.

The first case of alkaptonuria was described by Marcet in 1823; in 1858 Bödeker showed that the reducing substance was not glucose. Wolkow and Baumann in 1891 clearly established that the peculiar body in the urine was homogentisic acid (hydroquinone acetic acid):—



It is derived from the aromatic compounds—tyrosine and phenylalanine—in the protein food, and probably represents a stage in their catabolism in the body.

3. Cystinuria.

Cases of cystinuria are characterized by the deposits of cystine crystals in the form of hexagonal plates in the urine. The odour of hydrogen sulphide may become apparent when the urine decomposes.

The first case was that described by Wollaston in 1810, who obtained a cystine calculus from the bladder of a child. The phenomenon is believed to be the most common of the inborn errors, but is difficult of recognition.

It is also apparently due to incomplete oxidation of protein, especially the cystine unit. The diamines, putrescine and cadaverine, frequently accompany cystine in the urine; more rarely leucine and tyrosine.

4. Pentosuria.

The first case of the secretion of a pentose in the urine was described by Salkowski and Jastrowitz in 1892. Several cases have since been recorded. Though the urine reduced Fehling's solution, it did not ferment with yeast and was optically inactive. An osazone melting at 159° was isolated; glucosazone melts at 205° C. The presence of pentose can be confirmed by the phloroglucinol and orcinol tests (see p. 69).

6. PATHOLOGICAL URINES.

1. DIABETIC.

Diabetic urine contains glucose; β-hydroxybutyric acid, aceto-acetic acid and acetone are often present in diabetes mellitus.

The tests for glucose are Trommer's or Fehling's, Nylander's, fermentation, phenylhydrazine, polarization. (See under carbohydrates.)

Nylander's Test.

The reagent has the advantage over Fehling's in not being reduced by creatinine or uric acid. It is reduced by glycuronic acid. 1 c.c. reagent is added to 10 c.c. urine; on heating, the solution becomes yellow, brown, dark brown and metallic bismuth finally separates out. If the quantity of sugar be small, the urine darkens and, after standing for some time, a black deposit of metallic bismuth settles. The test will indicate 0·05 per cent. of glucose.

The Fermentation Test.

This succeeds well if the quantity of sugar be large, but if the quantity be small as indicated by Nylander's test it may not succeed. Allow the urine then to ferment for 24 to 28 hours and test again with Nylander's reagent. If Nylander's test is now negative, glucose was originally present. If positive, the reduction is probably due to other reducing substances, e.g. glycuronic acid, lactose.

The Phenylhydrazine Test.

This test is carried out preferably as follows: to 5 c.c. of urine add 2 c.c. of 50 per cent. acetic acid saturated with sodium acetate and two drops of phenylhydrazine. Evaporate down to 3 c.c., cool rapidly and again warm. Then allow to cool slowly. Crystals separate out even if there be a very small percentage of glucose. (Neumann.)

The Polarization Test.

The polarimeter distinguishes between glucose, fructose, and conjugated glycuronic acid, which is lævo-rotatory. If the urine be highly coloured, it is precipitated with lead acetate (10 c.c. of a 25 per cent. solution for every 50 c.c. of urine) and the filtrate is examined. The volumes must be known if the glucose be estimated by the polarimeter.

The estimation of glucose is generally carried out by Pavy's method (see estimation of carbohydrates).

Tests for Aceto-acetic Acid.

1. To some urine add dilute ferric chloride as long as a precipitate of ferric phosphate continues to form. Filter this off and to the filtrate add a few more drops of ferric chloride. If aceto-acetic acid be present the colour becomes like claret.

2. Acidulate some urine with sulphuric acid and shake it up with ether. Pour off the ether into another test tube and shake it with ferric chloride solution. A red colour is produced if aceto-acetic acid be present.

3. On heating with dilute alkali or acid, the aceto-acetic acid is decomposed yielding acetone; this may be detected by its odour. It is, however, detected by distilling and examining the first 20 c.c. of the distillate from 250 c.c. urine for acetone by the following tests:—

(*a*) Iodoform test: $NaOH + I$ in KI. Examine with microscope.

(*b*) Alcoholic solution of $I + NH_3$: iodoform and a black precipitate of nitrogen iodide, which disappears gradually leaving the iodoform.

(*c*) Legal's test. Add a few drops of freshly prepared sodium nitro-prusside solution and render alkaline with caustic soda. A deep red colour is formed. If acidified with acetic acid the colour becomes reddish-purple.

2. PROTEIN.

A. Coagulable Protein. ("Albumin.")

1. *Heat Coagulation.*

Add one-sixth of its volume of saturated sodium chloride to a little filtered urine and heat. The precipitate, which is formed, may consist of coagulated protein or earthy phosphates, or both. Now add 2 drops of 33 per cent. acetic acid to every 10 c.c. urine. The precipitate of earthy phosphates dissolves, whilst the coagulated protein separates out in flakes.

2. *Heller's Test.*

Pour some urine on to the surface of a little concentrated nitric acid in a test tube. If protein be present, a whitish ring will form at the junction. Also a reddish ring due to indigo-red and indigo-blue. If the urine be concentrated urea nitrate may separate out, but here the precipitate is obviously crystalline. Uric acid may also separate if a large quantity of urates be present. This can be prevented by diluting before testing with the nitric acid.

3. *Hydroferrocyanic acid:* see reactions of proteins.

4. *The colour reactions* cannot be applied directly to urine, but to the heat coagulum suspended in water. Try especially Millon's reaction and the biuret reaction; the coagulum in the latter case is dissolved in hot caustic soda.

5. *Characterization and Separation of the Protein.*

(*a*) *Albumin and Globulin.* Isolate the globulin by half saturating the urine with ammonium sulphate and filtering. Dissolve it in ·2 per cent. sodium chloride solution, acidify and heat.

Heat the filtrate after acidifying with acetic acid; the albumin is coagulated. Confirm the protein nature of the precipitates by the colour tests.

(*b*) *Proteose and Coagulable Protein.* Saturate the solution with ammonium sulphate (8 parts to 10 parts urine) and heat for a few seconds, so that the coagulable protein is coagulated. Filter. Extract the precipitate with alcohol to remove urobilin, which gives the biuret reaction. Then extract it with boiling water which dissolves the proteose. Test this solution with the biuret, xanthoproteic, and Millon's reactions.

6. *Estimation.*

The protein is estimated by Esbach's method (see under proteins, p. 151). The urine must be acidified with acetic acid if it is not acid and diluted till its specific gravity is 1·006-1·008.

The Bence-Jones Protein.

In rare cases of disease of the bone-marrow, a peculiar protein, the Bence-Jones protein, appears in the urine.

This protein is precipitated when the urine is heated to 50 to 60° C. and redissolves, more or less completely, according to the reaction and the amount of salt in the urine, when the heating is continued to the boiling-point.

The protein is not dialysable and can be precipitated by adding double the volume of saturated ammonium sulphate solution or alcohol. The exact nature of the body is unknown, but it has resemblances to globulin and proteose and yields the same amino acids on hydrolysis.

3. BLOOD.

The appearance of the urine may be reddish. Centrifuge the urine to separate corpuscles and examine these with the microscope.

Try the guaiac reaction (p. 213).

Examine with the spectroscope.

Prepare hæmochromogen by boiling with caustic soda, cooling and reducing with ammonium sulphide; this pigment shows the absorption bands when the other blood pigments do not. See under hæmoglobin.

The pigment can be precipitated with the earthy phosphates by caustic soda.

4. BILE.

Filter the urine through paper and perform Gmelin's reaction.
Perform Huppert's reaction.
} See under bile.

Pour some tincture of iodine carefully upon some urine in a test tube. At the junction of the liquids a green ring appears.

Also test for bile acids by:—

(1) Pettenkofer's test (after concentration if necessary).

(2) Hay's test.

(3) Oliver's test.

PRESSURE OF AQUEOUS VAPOUR (REGNAULT).

Temperature C.	Pressure in Mm. of Mercury.	Temperature C.	Pressure in Mm. of Mercury.
0·0	4·6	15·0	12·7
0·5	4·8	15·5	13·1
1·0	4·9	16·0	13·5
1·5	5·1	16·5	14·0
2·0	5·3	17·0	14·4
2·5	5·5	17·5	14·9
3·0	5·7	18·0	15·4
3·5	5·9	18·5	15·8
4·0	6·1	19·0	16·3
4·5	6·3	19·5	16·9
5·0	6·5	20·0	17·4
5·5	6·8	20·5	17·9
6·0	7·0	21·0	18·5
6·5	7·2	21·5	19·1
7·0	7·5	22·0	19·7
7·5	7·8	22·5	20·3
8·0	8·0	23·0	20·9
8·5	8·3	23·5	21·5
9·0	8·6	24·0	22·2
9·5	8·9	24·5	22·9
10·0	9·2	25·0	23·5
10·5	9·5	25·5	24·3
11·0	9·8	26·0	25·0
11·5	10·1	26·5	25·7
12·0	10·5	27·0	26·5
12·5	10·8	27·5	27·3
13·0	11·2	28·0	28·1
13·5	11·5	28·5	28·9
14·0	11·9	29·0	29·8
14·5	12·3	29·5	30·7

LIST OF REAGENTS.

ACIDS.

	100 c.c. contain
Acetic acid, glacial, sp. gr. 1·06	111·1 gm. CH_3COOH
Acetic acid, dilute, $\frac{1}{2}$ normal (27 c.c. glacial acetic acid made up to 1 litre)	3·0 gm. CH_3COOH
Hydrochloric acid, concentrated, sp. gr. 1·16	36·6 gm. HCl
Hydrochloric acid, dilute, $\frac{2}{1}$ normal (200 c.c. conc. acid made up to 1 litre)	7·3 gm. HCl
Hydrochloric acid, dilute, $\frac{1}{10}$ normal (10 c.c. conc. acid made up to 1 litre)	0·36 gm. HCl
Nitric acid, fuming, sp. gr. 1·40 (about 65 per cent.)	
Nitric acid, concentrated, sp. gr. 1·42	99·1 gm. HNO_3
Nitric acid, dilute, $\frac{2}{1}$ normal (125 c.c. conc. acid made up to 1 litre)	12·6 gm. HNO_3
Sulphuric acid, concentrated, sp. gr. 1·84	175·9 gm. H_2SO_4
Sulphuric acid, dilute, $\frac{2}{1}$ normal (56 c.c. conc. sulphuric acid made up to 1 litre)	9·8 gm. H_2SO_4
Sulphuric acid, dilute, $\frac{1}{10}$ normal (2·8 c.c. conc. sulphuric acid made up to 1 litre)	0·5 gm. H_2SO_4

62·5
31

ALKALIES.

Ammonia, concentrated, sp. gr. ·880	31·0 gm. NH_3
Ammonia, dilute, $\frac{2}{1}$ normal (105 c.c. conc. ammonia made up to 1 litre)	3·4 gm. NH_3
Barium hydroxide, $\frac{1}{2}$ normal (40 gm. $Ba(OH)_2 . 8H_2O$ dissolved in water and made up to 1 litre)	4·2 gm. $Ba(OH)_2$
Sodium hydroxide, sp. gr. 1·34 (410 gm. 98 per cent. caustic soda dissolved in water and made up to 1 litre) (426 gm. 94 per cent. caustic soda dissolved in water and made up to 1 litre)	40 gm. NaOH
Sodium hydroxide, dilute, $\frac{2}{1}$ normal (82 gm. 98 per cent. caustic soda dissolved in water and made up to 1 litre) (85 gm. 94 per cent. caustic soda dissolved in water and made up to 1 litre)	8·0 gm. NaOH
Sodium hydroxide, dilute, $\frac{1}{10}$ normal (4·1 gm. 98 per cent. caustic soda dissolved in water and made up to 1 litre) (4·2 gm. 94 per cent. caustic soda dissolved in water and made up to 1 litre)	0·4 gm. NaOH

SALT SOLUTIONS.

	100 c.c. contain
Ammonium chloride, $\frac{2}{1}$ normal (105 gm. NH_4Cl dissolved in water and made up to 1 litre)	10·5 gm.
Ammonium carbonate, $\frac{1}{1}$ normal (48 gm. $(NH_4)_2CO_3$ dissolved in water and made up to 1 litre)	4·8 gm.
62 ½ Ammonium molybdate (124 gm. $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ dissolved in cold water and made up to 1 litre)	11·5 gm.
Ammonium nitrate, $\frac{10}{1}$ normal (800 gm. NH_4NO_3 dissolved in water and made up to 1 litre)	80 gm.
Ammonium sulphide, $\frac{2}{1}$ normal	6·8 gm.
Barium chloride, $\frac{1}{1}$ normal 61 (122 gm. $BaCl_2 \cdot 2H_2O$ dissolved in water and made up to 1 litre)	10·4 gm.
Calcium chloride, $\frac{1}{2}$ normal (55 gm. $CaCl_2 \cdot 6H_2O$ dissolved in water and made up to 1 litre)	2·7 gm.
Copper sulphate (Fehling's solution A), about $\frac{1}{2}$ normal (69·2 gm. $CuSO_4 \cdot 5H_2O$ dissolved in water and made up to 1 litre)	4·0 gm.
Ferric chloride, $\frac{1}{2}$ normal (27 gm. dissolved in water and made up to 1 litre)	2·7 gm.
Iodine solution, $\frac{1}{10}$ normal (13 gm. iodine dissolved in 30 gm. KI in 250 c.c. water and made up to 1 litre)	1·3 gm. 1·3 62·8
Lead acetate, $\frac{1}{2}$ normal (95 gm. $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ dissolved in water and made up to 1 litre)	8·1 gm.
Lead acetate, basic, $\frac{1}{2}$ normal (56 gm. litharge dissolved in 95 gm. lead acetate in about 800 c.c. water and then made up to 1 litre)	13·6 gm.
Potassium bichromate, $\frac{1}{2}$ normal (24·55 gm. $K_2Cr_2O_7$ dissolved in water and made up to 1 litre)	2·4 gm.
Potassium chloride, $\frac{1}{1}$ normal (104·5 gm. KCl dissolved in water and made up to 1 litre)	10·4 gm.
Potassium ferrocyanide, $\frac{1}{2}$ normal (53 gm. $K_4Fe(CN)_6 \cdot 3H_2O$ dissolved in water and made up to 1 litre)	4·6 gm.
Potassium ferricyanide, $\frac{2}{1}$ normal (sat. sol.) (44 gm. $K_3Fe(CN)_6$ dissolved in water and made up to 1 litre)	4·4 gm.
Potassium oxalate, $\frac{1}{10}$ normal (8·3 gm. $K_2C_2O_4$ dissolved in water and made up to 1 litre)	0·8 gm.
Potassium permanganate, $\frac{1}{20}$ normal (1·581 gm. $KMnO_4$ dissolved in water and made up to 1 litre)	1·6 gm.
Potassium thiocyanate, $\frac{1}{10}$ normal (97·3 gm. KSCN dissolved in water and made up to 1 litre)	0·97 gm.
Mercuric chloride, $\frac{1}{2}$ normal (68 gm. $HgCl_2$ dissolved in water and made up to 1 litre)	6·8 gm.

	100 c.c. contain
Sodium carbonate, $\frac{1}{2}$ normal (71·5 gm. $Na_2CO_3 \cdot 10H_2O$ dissolved in water and made up to 1 litre)	2·6 gm.
Sodium chloride, $\frac{2}{1}$ normal (117 gm. NaCl dissolved in water and made up to 1 litre)	11·7 gm.
Silver nitrate, $\frac{1}{10}$ normal (17 gm. $AgNO_3$ dissolved in water and made up to 1 litre)	1·7 gm.
Sodium nitrite, $\frac{1}{10}$ normal (6·9 gm. $NaNO_2$ dissolved in water and made up to 1 litre)	0·7 gm.
Sodium phosphate, $\frac{1}{1}$ normal (119·5 gm. $Na_2HPO_4 \cdot 12H_2O$ dissolved in water and made up to 1 litre)	4·7 gm.
Uranium acetate, $\frac{1}{10}$ normal (21·3 gm. $UrO_2(C_2H_3O_2)_2 \cdot 2H_2O$ dissolved in water and made up to 1 litre) (1 c.c. = 0·00355 gm. P_2O_5)	2·1 gm.
Uranium nitrate, $\frac{1}{10}$ normal (25·18 gm. $UrO_2(NO_3)_2 \cdot 6H_2O$ dissolved in water and made up to 1 litre) (1 c.c. = 0·00355 gm. P_2O_5)	2·5 gm.

58·5
29·25

SOLIDS.

Ammonium carbonate.
Ammonium chloride.
Ammonium sulphate.
Barium carbonate.
Bismuth subnitrate.
Borax.
Bleaching powder. }
Calcium hypochlorite. }
Calcium carbonate.
Calcium chloride (dry).
Calcium sulphate (plaster of Paris).
Copper carbonate.
Copper oxide.
Copper turnings.
Ferrous sulphate.
Fusion mixture (3 parts KNO_3, 1 part Na_2CO_3).
Lime.
Litharge.
Magnesium oxide.
Magnesium powder.
Magnesium sulphate.
Phosphorus pentachloride.
Phosphorus pentoxide.
Potassium bichromate.
Potassium carbonate.
Potassium oxalate.
Potassium sulphate (acid).
Potassium permanganate.
Soda, caustic.
Soda, lime.
Sodium acetate, crystal.
Sodium acetate, fused.
Sodium carbonate (dry).
Sodium chloride.
Sodium (metallic).
Sodium nitrite.
Sodium nitroprusside.
Sodium sulphate.
Stannous chloride.
Sulphur, flowers of.
Zinc carbonate.

SPECIAL REAGENTS.

Acid sodium acetate.	100 gm. sodium acetate 30 c.c. glacial acetic acid are dissolved in water and made up to 1 litre.
Alcoholic caustic soda.	20 gm. sodium, or 20 gm. caustic soda are dissolved in alcohol and made up to 1 litre.
Ammonium sulphate solution (*sat.*).	780 gm. ammonium sulphate are dissolved in water and made up to 1 litre.
Barfoed's reagent.	66 gm. cupric acetate 10 c.c. glacial acetic acid are dissolved in water and made up to 1 litre.
Bromine water.	25 c.c. bromine in 1000 c.c. water.
Brücke's reagent.	50 gm. potassium iodide in 500 c.c. water are saturated with mercuric iodide (120 gm.) and made up to 1 litre.
Esbach's reagent.	10 gm. picric acid 10 gm. citric acid are dissolved in water and made up to 1 litre.
Fehling's solution.	Equal volumes of A and B. A. 69·2 gm. copper sulphate are dissolved in water and made up to 1 litre. B. 346 gm. Rochelle salt (NaK tartrate) 120 gm. caustic soda are dissolved in water and made up to 1 litre.
Folin's reagent (*uric acid*).	500 gm. ammonium sulphate 5 gm. uranium acetate 6 c.c. glacial acetic acid are dissolved in 650 c.c. water. The volume is about 1 litre.
Formalin.	Commercial 40 per cent. solution of formaldehyde.
Glyoxylic acid solution.	10 gm. magnesium powder are covered with water; 250 c.c. sat. oxalic acid solution are added slowly, and the solution kept cool. The magnesium oxalate is filtered off and the solution is acidified with acetic acid and made up to 1 litre.
Guaiacum tincture.	1 gm. of guaiacum is dissolved in 100 c.c. alcohol.
Gunzberg's reagent.	4 gm. phloroglucin 2 gm. vanillin are dissolved in 100 c.c. absolute alcohol.
Hydrogen peroxide.	10 vols. commercial.
Iron alum solution (*sat.*).	300 gm. iron alum in 1 litre water.
Ling's indicator.	1·5 gm. ammonium thiocyanate 1·0 gm. ferrous ammonium sulphate are dissolved in 10 c.c. water at 45° and cooled. 5 c.c. conc. hydrochloric acid are then added. The solution is decolorized by adding a small quantity of zinc dust.
Magnesia mixture.	55 gm. magnesium chloride 70 gm. ammonium chloride 125 c.c. ammonia (sp. gr. ·880) are dissolved in water and made up to 1 litre.
Magnesium sulphate solution (*sat.*).	600 gm. cryst. magnesium sulphate are dissolved in water and made up to 1 litre.
Mercuric nitrate.	10 gm. mercuric nitrate are dissolved in water and made up to 1 litre.
Millon's reagent.	400 gm. mercury (= 30 c.c.) are dissolved in 570 c.c. conc. nitric acid. The solution is then diluted with two volumes of water.

α-Naphthol solution.	144 gm. α-naphthol are dissolved in alcohol and made up to 1 litre.
Nylander's solution.	40 gm. Rochelle salt 20 gm. bismuth subnitrate are dissolved in 1000 c.c. caustic soda (8 per cent.).
Obermayer's reagent.	4 gm. ferric chloride are dissolved in 1 litre conc. hydrochloric acid.
Oil of turpentine.	Commercial.
Oxalic acid solution (sat.).	100 gm. oxalic acid are dissolved in 1 litre of water.
Pavy's solution.	120 c.c. Fehling's solution 300 c.c. ammonia (sp. gr. ·880) are made up to 1 litre with water.
Phenol solution.	20 gm. carbolic acid are dissolved in 1 litre of water.
Phosphotungstic acid solution.	50 gm. phosphotungstic acid 30 c.c. conc. sulphuric acid are dissolved in water and made up to 1 litre.
Picric acid solution (sat.).	12 gm. picric acid are dissolved in water and made up to 1 litre.
Sodium bisulphite solution (sat.).	600 gm. sodium bisulphite are dissolved in water and made up to 1 litre.
Sodium chloride solution (sat.).	370 gm. sodium chloride are dissolved in water and made up to 1 litre.
Sodium fluoride solution.	20 gm. sodium fluoride are dissolved in water and made up to 1 litre.
Sodium hypobromite solution.	25 c.c. bromine are added to 100 gm. caustic soda dissolved in 250 c.c. water.
Sodium sulphate solution: sp. gr. 1030.	40 gm. sodium sulphate are dissolved in 520 c.c. water.
sp. gr. 1035.	45 gm. ,, ,, ,, 510 c.c. ,,
sp. gr. 1040.	50 gm. ,, ,, ,, 500 c.c. ,,
sp. gr. 1045.	55 gm. ,, ,, ,, 500 c.c. ,,
sp. gr. 1050.	70 gm. ,, ,, ,, 500 c.c. ,,
sp. gr. 1055.	85 gm. ,, ,, ,, 500 c.c. ,,
sp. gr. 1060.	90 gm. ,, ,, ,, 500 c.c. ,,
sp. gr. 1065.	95 gm. ,, ,, ,, 500 c.c. ,,
sp. gr. 1070.	100 gm. ,, ,, ,, 500 c.c. ,,
Stoke's reagent.	30 gm. ferrous sulphate 20 gm. tartaric acid are dissolved in water and made up to 1 litre. When required for use, strong ammonia is added until the precipitate first formed is redissolved.
Tannic acid solution.	100 gm. tannic acid 25 gm. sodium acetate 25 gm. sodium chloride 50 c.c. glacial acetic acid are dissolved in water and made up to 1 litre.
Tartaric acid solution (sat.).	750 gm. tartaric acid are dissolved in water and made up to 1 litre.
Trichloracetic acid solution.	100 gm. trichloracetic acid are dissolved in water and made up to 1 litre.
Uffelmann's reagent.	Ferric chloride solution is added to 2 per cent. phenol solution till it is of a violet colour.

ORGANIC REAGENTS.

Acetic anhydride.
Acetone.
Alcohol.
Amyl alcohol.
Benzoyl chloride.
Chloroform.
Ether.
Glycerol.
Orcinol.
Petroleum.
Phenylhydrazine.
Phenylhydrazine hydrochloride.
Phloroglucinol.
Resorcinol.
Toluene.

INDICATORS.

Alizarin red.	10 gm. sodium alizarin sulphonate are dissolved in water and made up to 1 litre = 1 per cent.
Cochineal tincture.	5 gm. cochineal are extracted with 150 c.c. alcohol + 100 c.c. water for several days; the solution is then filtered.
Congo red.	1 gm. congo red is dissolved in water and made up to 1 litre = 0·1 per cent.
Litmus.	10 gm. litmus are finely powdered and extracted with 50 c.c. hot water. The blue liquid is decanted and made up to 1 litre = 1 per cent.
Methyl orange.	1 gm. methyl orange is dissolved in 500 c.c. alcohol and made up to 1 litre with water = 0·1 per cent.
Methyl violet.	1 gm. methyl violet is dissolved in water and made up to 1 litre = 0·1 per cent.
Phenolphthalein.	10 gm. phenolphthalein are dissolved in alcohol and made up to 1 litre with alcohol = 1 per cent.

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